5z d PCT/PTO 29 JAN 2001

(1390 REV. 5-93) US DEPT. OF COMMERCE PATENT & TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER
108366

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO.
(if known, sec 37 C.F.R.1.5)

09/744679

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/IL99/00079	February 5, 1999	August 7, 1998

TITLE OF INVENTION
METHOD FOR TREATMENT OF INVASIVE CELLS

APPLICANT FOR DO/EO/US
Rachel BAR-SHAVIT

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)

6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 11. to 16. below concern other document(s) *or* information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.

    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☒ Entitlement to small entity status is hereby asserted.

16. ☐ Other items or information:

525 Rec'd PCT/PTO 29 JAN 2001

U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5) 09/744679	INTERNATIONAL APPLICATION NO. PCT/IL99/00079	ATTORNEY'S DOCKET NUMBER 108366

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO ....$860.00 International preliminary examination fee paid to USPTO (37 CFR1.482) .....$690.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) .....$710.00 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....$1,000.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....$ 100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	19 - 20 =		X $ 18.00	$	
Independent Claims	5 - 3 =	2	X $ 80.00	$160.00	
Multiple dependent claim(s)(if applicable)			+ $270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$1,020.00	
Reduction by 1/2 for filing by small entity, if applicable. -				$510.00	
SUBTOTAL =				$510.00	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 month from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$510.00	
			Amount to be refunded	$	
			Charged	$	

a. ☒ Check No. 115823 in the amount of $510to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Director is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 15-0461. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
OLIFF & BERRIDGE, PLC
P.O. Box 19928
Alexandria, Virginia 22320

NAME: James A. Oliff
REGISTRATION NUMBER: 27,075

NAME: Joel S. Armstrong
REGISTRATION NUMBER: 36,430

(1390 Rev.10-00)

09/744679
525 Rec'd PCT/PTO 29 JAN 2001

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Rachel BAR-SHAVIT

Application No.: U.S. National Stage of PCT/IL99/00079

Filed: January 29, 2001 Docket No.: 108366

For: METHOD FOR TREATMENT OF INVASIVE CELLS

PRELIMINARY AMENDMENT

Director of the U.S. Patent and Trademark Office
Washington, D. C. 20231

Sir:

Prior to initial examination, please amend the above-identified application as follows:

IN THE CLAIMS:

Please replace claim 18 as follows:

18. (Amended) A method according to claim 17 wherein said antisense molecule is administrated to a trophoblast cell.

REMARKS

Claims 1-19 are pending. Claim 18 is amended to eliminate an incorrect dependency. Prompt and favorable consideration on the merits is respectfully requested.

The attached Appendix includes marked-up copies of each rewritten paragraph (37 C.F.R. 1.121(b)(iii)) and claim (37 C.F.R. 1.121(c)(ii)).

Respectfully submitted,

James A. Oliff
Registration No. 27,075

Joel S. Armstrong
Registration No. 36,430

Enclosure:
Appendix

JAO:JSA/kaf
Date: January 29, 2001

OLIFF & BERRIDGE, PLC
P.O. Box 19928
Alexandria, Virginia 22320
Telephone: (703) 836-6400

DEPOSIT ACCOUNT USE AUTHORIZATION
Please grant any extension necessary for entry;
Charge any fee due to our Deposit Account No. 15-0461

APPENDIX

Changes to Claims:

The following is a marked-up version of the amended claim: 18

18. A method according to claim ~~18~~ 17 wherein said antisense molecule is administrated to a trophoblast cell.

PTO/PCT Rec'd 29 AUG 2001

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Rachel BAR-SHAVIT BOX: SEQUENCE

Application No.: 09/744,679

Filed: April 11, 2001 Docket No.: 108366

For: METHOD FOR TREATMENT OF INVASIVE CELLS

SUPPLEMENTAL PRELIMINARY AMENDMENT

Director of the U.S. Patent and Trademark Office
Washington, D. C. 20231

Sir:

In reply to the Notification of a Defective Response mailed May 21, 2001, please amend the above-identified application as follows:

IN THE DRAWINGS:

Please replace Figure 10 as set forth in the attached Request for Approval of Drawing Correction.

IN THE SPECIFICATION:

At the end of the application, please insert the attached paper and computer-readable Sequence Listing.

Page 5, lines 14-19, delete current paragraphs and insert therefor:

1) The protease activated domains and hirudin binding domain:

	Nucleotides	
hPAR-1(ThR)	37-61.....	TLDPRSFLLRNPNDKYEPFWEDEEK (SEQ ID NO: 1)

hPAR-2 32-56.....SSKGRSLIGKVDGTSHVTGKGVTVE (SEQ ID NO: 2)

hPAR-3 34-57.....TLPIKTFRGAPPN SFEEFPFSALE (SEQ ID NO: 3)

hPAR-4 28-52.....LPAPRGYPGQVCANDSDTHELPDSS (SEQ ID NO: 4)

Page 6, lines 1-3, delete current paragraphs and insert therefor:

Fig. 1 shows the DNA (SEQ ID NO: 5) and amino acid sequence (SEQ ID NO: 6) of human ThR **[1]**.

Fig. 2 shows the DNA sequence of an antisense cDNA of ThR (SEQ ID NO: 7).

Fig. 3 shows the location of the ThR antisense in the pcDNA III vector.

Page 7, lines 24-26, delete current paragraphs and insert therefor:

Fig. 9 shows the DNA sequence of PAR-2 (SEQ ID NO: 8).

Fig. 10 shows the DNA sequence of PAR-3 (SEQ ID NO: 9).

Fig. 11a shows the DNA sequence of PAR-4 (SEQ ID NO: 10).

Fig. 11b shows the amino acid sequence of PAR-4 (SEQ ID NO: 11).

Page 15, lines 4-20, delete current paragraphs and insert therefor:

To analyze the impact of reduced ThR expression in the highly metastatic cells, MDA-435 breast carcinoma cells were transfected with an antisense ThR cDNA (SEQ ID NO: 7) mammalian expression vector containing ThR cDNA in an antisense orientation under the control of the Cytomegalovirus (CMV) promoter (see Figs. 2 and 3). The vector alone was used as a control. Western blot analysis of ThR protein levels showed a marked reduction in the antisense transfected cells (Fig. 8, lane A) as compared to vector alone (lane B) or untreated MDA-435 cells (lane C). When the antisense transfected cells were tested in the Matrigel invasion assay, the otherwise aggressively invading cells showed a markedly reduced level of invasion, similar to that of the non-metastatic breast carcinoma cell line MCF-7 (Fig. 8, E&F). Transfection with the vector alone had no effect on the invasion

properties and the transfected cells migrated effectively through the Matrigel layer (D), similar to the metastatic MDA-435 cells (A).

Similar antisense molecules may be prepared from other members of the PAR family, such as PAR-2 (SEQ ID NO: 8) (Fig. 9), PAR-3 (SEQ ID NO: 9) (Fig. 10) and PAR-4 (SEQ ID NO: 10) (Fig. 11a).

REMARKS

Claims 1-19 are pending. The attached Appendix includes marked-up copies of each rewritten paragraph (37 C.F.R. §1.121(b)(1)(iii)).

The attached paper copy and computer-readable copy of the Sequence Listing are submitted in compliance with 37 C.F.R. §§1.821-1.825. The contents of the paper copy and the computer-readable copy of the Sequence Listing are the same.

Support for the information provided in the Sequence Listing can be found in the specification at, for example, page 5 and in Figures 1, 2, 9 and 11, and in new Figure 10 filed herewith. Figure 10 is being replaced in order to correct an inadvertent error that was introduced into the specification. In particular, current Figure 10 recites the murine DNA sequence of PAR-3, rather than the human DNA sequence of PAR-3, which was clearly intended. New Figure 10 recites the human DNA sequence of PAR-3. The human DNA sequence of PAR-3 was known in the art at the time the present application was filed. As evidence of this, attached is a printout of Accession No. U92971 of the NCBI database indicating that the human DNA sequence of PAR-3 was known in the art. Thus, including this sequence in Figure 10 does not introduce new matter into the specification. As a result, no new matter is added in the Sequence Listing.

Early and favorable consideration on the merits is respectfully requested.

Respectfully submitted,

James A. Oliff
Registration No. 27,075

Melanie L. Mealy
Registration No. 40,085

JAO:MLM/jca

Attachments:
Appendix
NCBI Database Printout
Sequence Listing (paper and computer-readable copies)
Request for Approval of Drawing Correction

Date: August 29, 2001

OLIFF & BERRIDGE, PLC
P.O. Box 19928
Alexandria, Virginia 22320
Telephone: (703) 836-6400

DEPOSIT ACCOUNT USE AUTHORIZATION
Please grant any extension necessary for entry;
Charge any fee due to our
Deposit Account No. 15-0461

FAX RECEIVED
AUG 29 2001
TECH CENTER 1600/2900

APPENDIX

Changes to Specification:

A Sequence Listing is added.

Page 5, lines 14-19:

1) The protease activated domains and hirudin binding domain:

	Nucleotides	
hPAR-1(ThR)	37-61.....	TLDPRSFLLRNPNDKYEPFWEDEEK (SEQ ID NO: 1)
hPAR-2	32-56.....	SSKGRSLIGKVDGTSHVTGKGVTVE (SEQ ID NO: 2)
hPAR-3	34-57.....	TLPIKTFRGAPPN SFEEFPFSALE (SEQ ID NO: 3)
hPAR-4	28-52.....	LPAPRGYPGQVCANDSDTHELPDSS (SEQ ID NO: 4)

Page 6, lines 1-3:

Fig. 1 shows the DNA (SEQ ID NO: 5) and amino acid sequence (SEQ ID NO: 6) of human ThR **[1]**;

Fig. 2 shows the DNA sequence of an antisense cDNA of ThR (SEQ ID NO: 7);

Fig. 3 shows the location of the ThR antisense in the pcDNA III vector;

Page 7, lines 24-26:

Fig. 9 shows the DNA sequence of PAR-2 (SEQ ID NO: 8);

Fig. 10 shows the DNA sequence of PAR-3 (SEQ ID NO: 9);

Fig. 11a shows the DNA sequence of PAR-4 (SEQ ID NO: 10);

Fig. 11b shows the amino acid sequence of PAR-4 (SEQ ID NO: 11). ~~and~~

Page 15, lines 4-20:

To analyze the impact of reduced ThR expression in the highly metastatic cells, MDA-435 breast carcinoma cells were transfected with an antisense ThR cDNA. (SEQ ID NO: 7) mammalian expression vector containing ThR cDNA in an antisense orientation under the control of the Cytomegalovirus (CMV) promoter (see Figs. 2 and 3).

The vector alone was used as a control. Western blot analysis of ThR protein levels showed a marked reduction in the antisense transfected cells (Fig. 8, lane A) as compared to vector alone (lane B) or untreated MDA-435 cells (lane C). When the antisense transfected cells were tested in the Matrigel invasion assay, the otherwise aggressively invading cells showed a markedly reduced level of invasion, similar to that of the non-metastatic breast carcinoma cell line MCF-7 (Fig. 8, E&F). Transfection with the vector alone had no effect on the invasion properties and the transfected cells migrated effectively through the Matrigel layer (D), similar to the metastatic MDA-435 cells (A).

Similar antisense molecules may be prepared from other members of the PAR family, such as PAR-2 (SEQ ID NO: 8) (Fig. 9), PAR-3 (SEQ ID NO: 9) (Fig. 10) and PAR-4 (SEQ ID NO: 10) (Fig. 11a).

24/prts

09/744679

WO 00/08150

PCT/IL99/00079

525 Rec'd PCT/PTO 29 JAN 2001

METHOD FOR TREATMENT OF INVASIVE CELLS

FIELD OF THE INVENTION

This invention relates to the therapeutic use of molecules associated with protease activated receptors.

BACKGROUND OF THE INVENTION

References referred to by bracketed numbers in the body of the specification are listed at the end of the specification before the claims.

The process by which epithelial cells become invasive is complex and has yet to be fully elucidated. One example of this process is observed in metastatic tumors. Another example of epithelial cells becoming invasive occurs during normal human embryonic development, in which the cytotrophoblasts (i.e. the fetal cells on the front line of the placenta) invade the uterus, as part of their normal differentiation program and successful implantation.

The physiologic invasiveness of cytotrophoblasts closely resembles that of malignant cells, sharing many common features. Tumor invasion and metastasis involve, among other alterations, proteolytic modification of basement membranes and extracellular matrices (ECMs). Cancer cells have to detach from their primary location, encounter basement membranes (i.e. during extravasation of blood or lymphatic vessels), and disseminate through the circulation to establish new cellular colonies at distant sites. Therefore, the process of cell invasion involves a well-orchestrated sequence of events including integrin activation, cell migration and proteolytic degradation of specific barrier components. This enzymatic cleavage is highly regulated, since extensive proteolysis could impede the invasive process by degrading essential

matrix components required for the transmission of survival and cell shape signals, through contacts with the basement membrane. Localized proteolysis directed to discrete regions of the cell surface may facilitate cellular invasion.

The thrombin-receptor (ThR) is a seven transmembrane domain G-coupled protein, belonging to the protease-activated receptor (PAR) family **[1]**. Recently, two other members of this family (PAR-2 and PAR-3) have been identified **[2-4]**, and a fourth member (PAR-4) has also been described **[19]**. Unlike most cellular growth factor receptors, the activation of these receptors does not require formation of the traditional ligand-receptor complex. Instead, the receptor serves as a substrate for proteolytic digestion, yielding an irreversible form of activated cell surface protein to convey further cell signaling.

Applicant's co-pending Israel Patent Application No. 114890, whose contents are incorporated herein by reference, discloses that a direct correlation exists between ThR level of expression in tumor cells and their degree of invasiveness. This finding was used to develop a diagnostic method for evaluating the metastatic tendency of tumor cells by following the expression of the ThR gene.

U.S. 5,352,664 to Carney, *et al*, describes thrombin-derived polypeptides which are capable of selectively stimulating or inhibiting thrombin receptor occupancy signals. Carney suggests that the inhibitory polypeptides may be used in preventing metastasis and angiogenesis. No supporting data is disclosed.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method for treating metastatic tumors.

It is a further object of the present invention to provide a method for treating irregularities in physiological placental development.

The present invention is based on the surprising finding that interfering with the expression of PAR proteins of an invasive cell affects its degree of invasiveness. The interference may be realized at the DNA (gene) level, at the mRNA level, and/or at the protein (receptor) level. Interference at the DNA level may be achieved by use of gene therapy methods; interference at the mRNA level may be achieved by use of antisense molecules; and interference at the protein level may be achieved by use of specific antibodies.

The PAR protein may be any member of the PAR family such as, for example but not limited to, ThR, PAR-2, PAR-3 and PAR-4.

In a first aspect of the invention, the invasive cells are pathological cells such as metastatic tumor cells. Thus, in this aspect of the invention, there is provided a method for treating metastatic tumor cells of a subject comprising administrating to said subject an antisense molecule, said antisense molecule comprising a nucleotide sequence which is complementary to an RNA sequence of a PAR protein.

Also provided are antisense molecules and pharmaceutical compositions comprising them.

Further provided is a method for treating metastatic tumor cells of a subject comprising administrating to said subject an antibody molecule, said antibody molecule being capable of binding to a protease activated receptor (PAR) protein. The antibody molecule may be a polyclonal or monoclonal antibody, prepared by methods known *per se*.

In this aspect of the invention, the tumor cells will generally be of epithelial origin, which form solid carcinoma-type tumors. Examples of such epithelial tissues are breast, esophagus, kidney, prostate, ovary, melanoma and bladder tissue.

In a second aspect of the invention, the invasive cells are normal cells such as placental cells. As described above, ThR plays a role during cytotrophoblast invasion and implantation. The finding that ThR expression is associated with the invasiveness of placental tissue may be beneficial for

improved implantation of human embryo in the maternal uterus decidua. To date, the rate of spontaneous abortions is 8-12%, 50% of which are due to defects in proper implantation. It is even more striking in the I.V.F. procedure, where 40% of the overall cases result in failure. 90% of these failures are apparently due to implantation defects. Transfection of normal placenta with ThR and other PAR family genes may considerably improve implantation.

Thus, in this aspect of the invention, there is provided a method for the treatment of disorders involving the implantation of a placenta in a female subject comprising administrating to said subject an antisense molecule, said antisense molecule comprising a nucleotide sequence which is complementary to an RNA sequence of a PAR protein.

Also provided are antisense molecules and pharmaceutical compositions comprising them.

The synthesis of antisense molecules to known mRNA sequences is well known to the skilled artisan. In theory, based on Watson-Crick base pair formation, if an appropriate target can be identified, an antisense oligomer of more than 15 to 17 nucleotides in length would be expected to have a unique sequence relative to the entire human genome. A suitable oligomer should be able to interfere, in a sequence specific manner with the process of mRNA translation into protein **[9]**. The requirements for an antisense oligomer for therapeutic use are: (1) that it must be stable *in vivo; (2)* it must be able to enter the target cell; and (3) it must be able to interact with its cellular targets.

As oligomers possess little or no innate ability to diffuse across cell membranes, the cells must take them up through energy-dependent mechanisms. To resolve the problem of uptake, a large number of strategies have been employed in order to augment the rate of cellular internalization of nucleic acids and to increase the rate at which they pass through the endosomal membrane. These strategies include: (i) coupling oligomers to polycations such as polylysine **[10]**, polyethylamine **[11]** or others; (ii) use of transferin/polylysine-conjugated DNA in the presence of the capsid of a

replication-deficient adenovirus **[12]**; (iii) conjugation of oligonucleotides to fusogenic peptides **[13]** or to a peptide fragment of the homeodomain of the *Drosophila* antennapedia protein **[14]**; (iv) targeting of oligonucleotides to specific cell surface receptors, such as folate, asialoglycoprotein receptor and transferrin **[15]**, (v) conjugation to cholesterol **[16]**; and, most successfully (vi) complexation of oligonucleotides with cationic lipids [17] and GS 288 etofectin **[18]**.

Preferred antisense sequences are those designed to comprise sequences which hybridize to uniquely conserved regions in the PAR family of proteins. Conserved regions may be identified by comparing the nucleotide sequences of different members of the PAR family. For example, certain regions within the ThR sequence have 27% sequence similarity to PAR-3 and 28% similarity to PAR-2. Examples of conserved unique regions are:

1) The protease activated domains and hirudin binding domain:

	Nucleotides
hPAR-1(ThR)	37-61..... TLDPRSFLLRNPNDKYEPFWEDEEK
hPAR-2	32-56.....SSKGRSLIGKVDGTSHVTGKGVTVE
hPAR-3	34-57.....TLPIKTFRGAPPN SFEEFPFSALE
hPAR-4	28-52.....LPAPRGYPGQVCANDSDTHELPDSS

2) Second extracellular loop: located between transmembrane domains 4 & 5 and corresponding to residues: **ITTCHDV** which are conserved in PAR 1-3, while in PAR-4 only the three amino acids **CHD** are conserved.

3) The entire promoter region of the PAR family (i.e. 5′ cloned regions downstream to the ATG of PAR-1 and PAR-3). This region is likely to contain important regulatory sequences.

DETAILED DESCRIPTION OF THE DRAWINGS:

In order to understand the invention and to see how it may be carried out in practice, a preferred embodiment will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 shows the DNA and amino acid sequence of human ThR [1];

Fig. 2 shows the DNA sequence of an antisense cDNA of ThR;

Fig. 3 shows the location of the ThR antisense in the pcDNA III vector;

Fig. 4 illustrates ThR expression in human breast carcinoma cell lines. Total RNA isolated from human breast carcinoma cell-lines was analyzed by Northern blotting. The cell lines used were: MDA-435 (A), MDA-231 (B) and MCF-7 (C), as well as Ha-ras-transfected breast carcinoma cell lines, MCF10AT3B (D), MCF10AT (E) and MCF10A (F). The blots were probed with ^{32}P-labeled 250 base pair DNA, corresponding to ThR (upper part), or with ^{32}P-labeled β-actin DNA (lower part).

Fig. 5 illustrates immunocytochemical analysis of cell-associated ThR. Human breast carcinoma cell lines (MCF-7, MDA-231, and MDA-435) were cultured in 8-well chamber slides and analyzed for the presence of ThR. Specific staining of the receptor was obtained following incubation with affinity purified polyclonal anti ThR antiserum followed by biotin conjugated goat-anti-rabbit 'IgG antibodies and detected by extravidin incubation. Photographs of representative areas of MCF-7 (*a*), MDA-231 (*b*) and MDA-435 (*c*) cell monolayers are shown (x400).

Lower Panel. Western blot analysis of ThR. Western blot analysis of cell lysates (50μg/lane) of MCF-7 (A), MDA-231(B) and MDA-435 (C) cells. Specific protein band was detected following incubation with anti ThR antibodies and visualized by the ECL immunoblotting detection system according to the manufacturer's instructions.

Fig. 6 illustrates *in situ* hybridization of ThR mRNA in normal and cancerous breast tissue specimens. Hybridization with ThR riboprobes was performed on: Normal breast duct lobular units (A&*D*). Invasive duct carcinoma, (IDC) (antisense orientation, C; sense orientation, B). High grade DCIS of comedo type (antisense orientation, *E*; sense orientation, F). Low grade DCIS, solid type (G) and atypical intraductal hyperplasia (AIDH, H &*I*).

Detection of specifically hybridized mRNA to DIG-labeled probe was performed using anti-DIG-alkaline phosphatase conjugated antibodies (Boehringer Mannheim, Mannheim, Germany). These analyses represent at least 3 patients of each category.

Fig. 7 illustrates Matrigel invasion of breast carcinoma cell lines. The indicated cells (ZR-75, A; MCF-7, B; MDA-435, C; MDA-231, D; fibrocystic MCF10AT3B, E; fibrocystic MCF10A, F) were applied ($2x10^5$ cells/assay) to the upper compartment of Boyden chambers. Cell invasion through Matrigel coated filters was determined, as outlined in Materials and Methods, below.

Fig. 8 illustrates inhibition of MDA-435 Matrigel invasion by ThR antisense. MDA-435 cells were transiently transfected with pCDNAIII expression plasmid containing the antisense ThR of Fig. 2. The level of invasion was compared to untreated MDA-435 (A) and MCF-7 (B) cells. Control transfections of MDA-435 cells were performed in the presence of vector alone - (C) or DOTAP liposomes alone (Gibco -BRL) (D). Nearly confluent (60%) cells were treated with various concentrations of the plasmid: transfection with antisense ThR - 5 μg/plate (E), transfection with antisense ThR - 20 μg/plate (F). The invasion assay was performed as described under Materials and Methods, 72 h following transfection.

Lower panel. Western blot analysis of ThR antisense transfectants. MDA-435 cell lysates (50μg/lane) of ThR antisense transfectants (A) were applied on SDS-PAGE and the level of receptor protein was compared to cells transfected with vector alone (B) or untreated cells (C).

Fig. 9 shows the DNA sequence of PAR-2;

Fig. 10 shows the DNA sequence of PAR-3;

Fig. 11 shows the DNA sequence of PAR-4; and

Fig. 12 illustrates expression of ThR in first trimester human placenta. *In situ* hybridization analysis of ThR expression at 6-15 weeks of gestation. Placental tissue was obtained from elective termination of pregnancies by dilatation and curettage. Sections of 6 week placental tissue (A) and of 7, 8, 9

and 10 weeks of gestation (B-*E*, respectively), as visualized by ThR staining of cytotrophoblasts. No staining was observed at weeks 11 and 15 (F & *G*, respectively). Control hybridization (weeks 7 and 8) using sense orientation showed background staining (H & *I*, respectively).

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

Materials and Methods

Cells: The human breast carcinoma cell lines, MCF-7 (adenocarcinoma), MDA-MB-231 (adenocarcinoma), MDA-MB-435 (ductal carcinoma) and ZR-75-1 (carcinoma), were kindly provided by Dr. Robert Stern (Department of Pathology, University of California, San Francisco). The invasive properties of these breast cell lines were determined following injection of the cells into the mammary pads of nude mice with or without Matrigel **[5]**. Cells were cultured in DMEM (1g glucose/liter) containing 10% bovine calf serum. MCF10A (nearly-normal immortalized epithelial cells), MCF10AT cells (derived from human fibrocystic epithelium transfected with Ha-ras) and MCF10AT3B cells (derived from a 94-day third transplant generation of lesion in Beige /Nude mice, classified as grade 2), were kindly provided by Dr. F. R. Miller (Karamanos Cancer Institute, Meyer L. Prentiss Center, Detroit) and grown in RPMI-1640 containing 10% fetal calf serum (FCS). Tissue culture medium was supplemented with penicillin (50 U/ml) and streptomycin (50 μg/ml) (GIBCO-BRL, Gaithersburg, MD) and the cells were maintained at 37°C in a 10% CO_2 humidified incubator. Cells were dissociated with 0.05% trypsin/0.02% EDTA, 0.01M sodium phosphate (pH 7.4) solution (STV) and subcultured at a split ratio of 1:5.

Plasmids and transfection: The DNA and amino acid sequences of ThR are shown in Fig. 1 **[1]**. ThR in the antisense orientation (Fig. 2), consisting of 612 nucleotides (from (-)75 to (+)537 of Fig. 1) was prepared and inserted into the

eukaryotic expression plasmid, pcDNA III (Invitrogene, Carlsbad, CA) at the HindIII and EcoRI sites (Fig. 3). Antisense ThR cDNA was used for transient transfection experiments. Subconfluent (25-40%) MDA-435 breast cancer cells were grown in 60 mm culture dishes and a total of 5-20 μg of DNA and DOTAP - transfection reagent (10 μg DOTAP/μg DNA; 4.5 h incubation, Boehringer Mannheim, Mannheim, Germany) were used for transfection. Cells were assayed 48-72 h following transfection.

RNA Isolation and Northern blot analysis: RNA was prepared using TRI-Reagent (Molecular Research Center, Inc. Cincinnati) according to manufacturer's instructions. The RNA (20 μg of total RNA) was separated by electrophoresis through a 1.1% agarose gel containing 2 M formaldehyde, transferred to a nylon membrane (Hybond N^+; Amersham) and hybridized either to cDNA probes or PCR product radiolabeled by random primer extension with [α-^{32}P]Dct **[6]** for 24 h at 42°C. The membrane was washed twice for 30 min at room temperature with 2x SSC containing 2% SDS and 15 min at 50°C with 0.1x SSC, containing 0.1% SDS. The blots were exposed for 2-4 d at -70°C and the relative amounts of mRNA transcripts were analyzed by laser densitometry using an Ultroscan XL Enhanced Laser Densitometer and normalized relative to internal β-actin controls.

In situ *hybridization of human tumor and placenta biopsy specimens.* RNA probes were transcribed and labeled by T_7 RNA polymerase (for antisense orientation) or T_3 RNA polymerase (for sense control orientation) using DIG-UTP labeling mix (Boehringer Mannheim, Mannheim, Germany). Probes were labeled from plasmid containing 462 base pair fragments of the human ThR (pBhThR-462S) inserted into the EcoRI-HindIII site. Final concentration for hybridization was 1 μg/ml, according to the manufacturer's instructions for non radioactive *in situ* hybridization application. Hybridization was carried out

(overnight, 45°C) on paraffin embedded breast tissue sections (Department of Pathology, Hadassah University Hospital, Jerusalem) or placenta sequential sections. Slides were washed in 0.2xSSPE (3x 1 h) at 50°C and blocked by blocking reagent (Boehringer Mannheim, Mannheim, Germany). Detection was performed using AP-conjugated, anti-DIG antibodies (Fab-fragment, diluted 1:300; Boehringer Mannheim, Mannheim, Germany), overnight at room temperature. AP reaction was detected by NBT/BCIP reagents according to the manufacturer's instructions.

Immunohistochemistry: Tumor cells were cultured overnight at 37°C on eight chamber slides. The cells were fixed with 2% formaldehyde and 2% sucrose/PBS at room temperature for 30 min and permeabilized with 20 mM Hepes, pH 7.4, 300 mM sucrose, 50 mM NaCl, 3 mM $MgCl_2$ and 0.5% Triton X-100, for 4 min at 0°C. After rehydration with PBS, the cells were incubated (10 min, 24°C) with 3% H_2O_2 in PBS containing 10 mM glycine, 10 mg/ml BSA, followed by 30 min blocking with normal goat serum in PBS containing 1% BSA. Affinity purified rabbit-anti-human ThR antibodies were added (dilution 1:50-1:200) for 4 h at 4°C, followed by incubation (1 h, room temperature) with a second antibody goat-anti-rabbit IgG-Biotin conjugated and 1 h incubation with HRP-ExtraAvidin (1:200) (Sigma Immuno Chemicals, St. Louis, MO).

Antibodies: We have raised anti-ThR antibodies directed toward a synthetic peptide (thrombin- receptor activating peptide; TRAP) corresponding to residues Ser42-Lys51 (i.e. S-F-L-L-R-N-P-N-D-K). KLH conjugated peptide was injected to rabbits, and the immune serum was affinity purified. ELISA was performed on plates coated with the TRAP-peptide showing efficient positive identification at 1:25,600 dilution. Maximal response was obtained at 1:3,200

dilution. Monoclonal anti ThR Abs (mouse IgG1 clone IIaR-A) were used for Western blot analysis (Biodesign, ME, USA)

Western blotting analysis: Cells were dissolved in lysis buffer containing 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100 and protease inhibitors (5 μg/ml aprotinin, 1μM phenylmethylsulfonylfluoride and 10 μg/ml leupeptin) for 30 min at 4°C. After centrifugation at 10,000 g for 20 min at 4°C, the supernatants were transferred and the protein content was measured. Lysates (50 μg) were loaded and resolved on 10% SDS-PAGE followed by transfer to Immobilon-P membrane (Millipore, MA). Membranes were blocked and probed with anti-ThR antibodies (1:4000) in 1% BSA in 10 mM Tris-HCl (pH 7.5), 100 mM NaCl and 0.05% Tween-20). After washes, blots were incubated with the appropriate second antibodies and conjugated to horseradish peroxidase. Immunoreactive bands were detected by the enhanced chemiluminescence (ECL) reagent using luminol and p-cumaric acid (Sigma, St. Louis, Mo).

Placental tissue sections: Sections of placental tissue, 6-15 weeks of gestation, were obtained from elective termination of normal pregnancies by dilatation and curettage.

Matrigel invasion assay: Blind well chemotaxis chambers with 13 mm diameter filters were used for this assay. Polyvinylpyrrolidone-free polycarbonate filters, 8 μm pore size (Costar Scientific Co., Cambridge, MA), were coated with basement membrane Matrigel (25 μg/filter) as previously described [7]. Briefly, the Matrigel was diluted to the desired final concentration with cold, distilled water, applied to the filters, dried under a hood, and reconstituted with serum-free medium. Cells (2-3x10^5), suspended in DMEM containing 0.1% bovine serum albumin were added to the upper chamber.

Conditioned medium of 3T3 fibroblasts was applied as a chemoattractant and placed in the lower compartment of the Boyden chamber. Assays were carried out at 37°C in 5% CO_2. Over 90% of the cells attached to the filter after a 2h incubation. At the end of the incubation, the cells on the upper surface of the filter were removed by wiping with a cotton swab. The filters were fixed in methanol and stained with hematoxylin and eosin. Cells in various areas of the lower surface were counted and each assay was performed in triplicate. For chemotaxis studies, filters were coated with collagen type IV alone (5μg/filter) to promote cell adhesion. Cells were added to the upper chamber and conditioned medium was applied to the lower compartment.

Examples

Example I: ThR expression in breast carcinoma cell lines.

In a preliminary experiment, a panel of mammary carcinoma cells was surveyed for a possible correlation between the level of ThR expression and established degrees of metastasis (Fig. 4). The cell lines used included one near-normal diploid immortalized breast epithelial cell line (MCF10A) originating from fibrocystic disease, and 6 tumor cell lines exhibiting different doubling times, tumorigenicity and metastases in nude mice. Of these cell lines, MDA-435 (a highly metastatic cell line), and MCF10AT3B (ras transfected fibrocystic epithelium re-established several times from lesions formed in nude mice), were compared to medium metastatic (MDA-231 and MCF10AT, ras transfected fibrocystic cells), or carcinoma cells exhibiting no metastatic potential (ZR-75 and MCF-7 cells). As shown in Fig. 4, high levels of ThR mRNA were found in the highly aggressive cells (lanes A, D) as compared to moderate levels in MDA-231 and ras transfected fibrocystic cells (lanes B& E, respectively), and no expression in the non-metastatic MCF-7 and MCF10AT cells (lanes C&F, respectively). The mRNA levels were quantified by densitometric analysis and the ratio of ThR/β-actin in each lane was calculated. The ThR mRNA level in MDA-435 was 6 fold higher than in MDA-231 cells

(Fig. 4, lanes A vs B) and, as mentioned above, no detectable ThR was observed in MCF-7 cells (Fig. 4, lane C). A similar correlation between ThR level of expression and metastasis was obtained in Ha-ras transfected cells showing a 4 fold higher level in MCF10AT3B (obtained following ras-transfection and xenografting 3 times in mice) than in MCF10AT-ras transfected cells (Fig. 4, lanes D vs E). No detectable level of expression was observed in the fibrocystic, non-malignant, epithelial cells, MCF10A epithelial cells (Fig. 4, lane F).

Affinity purified rabbit-anti-human ThR antibodies were applied to detect the expression and localization of the receptor protein. Massive staining of MDA-231 and MDA-435 cells was observed (Fig. 5B&*C*, respectively), as opposed to little or no staining of MCF-7 cells (Fig. 5A). In parallel, Western blot analysis showed a distinct protein band of ThR in MDA-435 cells (Fig. 5, lower panel; lane C), somewhat reduced ThR level in MDA-231 (lower panel; lane B) and little or no protein in MCF-7 breast carcinoma cells (lower panel; lane A).

Collectively, these data demonstrate the preferential expression of ThR in metastatic breast carcinoma cell lines, but not in non-metastatic MCF-7 or MCF10A breast carcinoma cells, regardless of whether the mRNA or protein levels were evaluated.

Example 2: ThR expression in human breast tissue specimens.

ThR gene expression and localization *in vivo* was studied in formalin fixed paraffin embedded human breast carcinoma specimens as compared to normal mammary sections obtained from reduction mammoplasty. ThR expression was examined in primary breast tumors representing poor to benign prognosis. *In situ* hybridization analysis using a ThR RNA probe (corresponding to nucleotide nos. 320-570 of the sequence of Fig. 1) was performed with an archival set of paraffin embedded biopsy specimens. A total of 10 normal breast tissue specimens, and 8 specimens of infiltrating ductal carcinoma were analyzed. The invasive carcinoma specimens were selected

from typical infiltrating duct carcinoma of high nuclear grade with numerous atypical mitotic figures and with evidence of vascular invasion and lymph node metastases.

As demonstrated in Fig. 6, hybridization of a ThR antisense RNA probe to invasive duct carcinoma specimens resulted in strong positive staining localized specifically to the carcinoma cells (Fig. 6C). Weaker positive staining was noted in high-grade ductal carcinoma *in situ* (DCIS) of comedo-type (Fig. 6 E&*F*). In contrast, very little or no staining was observed in low-grade, solid type DCIS (Fig. 6G), and no staining was observed in premalignant atypical intraductal hyperplasia (AIDH) (Fig. 6 H&*I*) and in normal breast duct lobular units (Fig. 6 A&*D;* note that the high staining seen in the background is limited to the fibers, and is not seen in the epithelial cells). AIDH was distinguished from low grade DCIS, non-comedo type according to the diagnostic criteria of Dupont, Page and Rogers **[8]**. Expression was also noted in some cases of DCIS, in particular, high grade, comedo-type lesions. The low grade DCIS of solid type showed weak to no expression of ThR, while cases of AIDH, as well as normal breast tissue from reduction mammoplasty specimens did not show any expression of ThR.

Example 3: Antisense ThR inhibits metastatic breast carcinoma cell invasion.

To assess the invasion properties of aggressively metastatic breast carcinoma cells, the Matrigel *in vitro* invasion assay was applied. For this purpose, a reconstituted matrix of basement membrane was utilized to coat porous filters, in order to closely mimic natural barriers in a Boyden chamber. As a chemoattractant source, fibroblast conditioned medium was placed in the lower compartment [7]. The Matrigel invasion assay confirmed the expected differential metastatic properties of the carcinoma cell lines. High levels of invasion through Matrigel were obtained with MDA-435 and MDA-231 cells (Fig. 7, D&C). MCF10AT3B-ras transfected fibrocystic cells invaded the

Matrigel to a lower extent (Fig. 7, E), while no movement was detected with the MCF10AT, MCF-7, or ZR-75 non-metastatic cell lines (Fig. 7, F & A ,B, respectively).

To analyze the impact of reduced ThR expression in the highly metastatic cells, MDA-435 breast carcinoma cells were transfected with an antisense ThR cDNA. mammalian expression vector containing ThR cDNA in an antisense orientation under the control of the Cytomegalovirus (CMV) promoter (see Figs. 2 and 3). The vector alone was used as a control. Western blot analysis of ThR protein levels showed a marked reduction in the antisense transfected cells (Fig. 8, lane A) as compared to vector alone (lane B) or untreated MDA-435 cells (lane C). When the antisense transfected cells were tested in the Matrigel invasion assay, the otherwise aggressively invading cells showed a markedly reduced level of invasion, similar to that of the non-metastatic breast carcinoma cell line MCF-7 (Fig. 8, E&F). Transfection with the vector alone had no effect on the invasion properties and the transfected cells migrated effectively through the Matrigel layer (D), similar to the metastatic MDA-435 cells (A).

Similar antisense molecules may be prepared from other members of the PAR family, such as PAR-2 (Fig. 9), PAR-3 (Fig. 10) and PAR-4 (Fig. 11).

Example 4: ThR expression during placenta development.

Human embryo development depends on proper placentation and successful implantation. Trophoblast invasion through the uterine epithelium and deep into the stroma enables the establishment of the proper fetal-maternal interactions. Histological examination of placental biopsies during the first trimester (6-15 weeks), obtained from elective termination of pregnancies, showed a striking pattern of ThR temporal regulation. ThR mRNA levels were not detected up to 6 weeks of gestation (Fig. 12,A), increased markedly between 7-10 weeks (B-*E*), then fell precipitously at 11 weeks and thereafter (F*&G*). The staining was specific to ThR, since hybridization with ThR sense

orientation on placental biopsies taken on weeks 7 and 8, showed no staining (H&I, respectively). The receptor appeared localized to the cytotrophoblasts within the villi, and also, to some extent, in the syncytiotrophoblasts of the invading column.

REFERENCES

1. Vu, T-K., Hung, H.D.T., Wheaton V.I. & Coughlin, S.R. Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell* **64**, 1057-1068 (1991).

2. Nystedt, S., Emilsson, K., Wahlestedt, C. & Sundelin, J. Molecular cloning of a potential proteinase activated receptor. *Proc. Natl. Acad. Sci. USA* **91**, 9208-9212 (1994).

3. Nystedt, S., Emilsson, K., Larsson, A-K., Strombeck, B. & Sundelin, J. Molecular cloning and functional expression of the gene coding for the human proteinase-activated receptor 2. *Eur. J. Biochem* **232**, 84-89 (1995).

4. Ishihara, H. *et al.* Protease-activated receptor-3 is a second thrombin receptor in humans. *Nature* **386**, 502-506 (1997).

5. Giancotti, F.G. & Ruoslahti, E. Elevated levels of the $\alpha_5\beta_1$ fibronectin receptor supresses transformed phenotype of Chinese hamster ovary cells. *Cell* **60**, 849-859 (1990).

6. Feinberg, V. & Vogelstein, B. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem* **132**, 6-13 (1984).

7. Albini, A. *et al.* A rapid *in vitro* assay for quantitating the invasive potential of tumor cells. *Cancer Res.* **47**, 3239-3245 (1987).

8. Page, D.L. *et al.* Atypical hyperplastic lesions of the female breast: a long term follow-up study. *Cancer* **55**, 2698-2708 (1985).

9. Toulme, J. and Helene, C. (1988) Gene **72**, 51.

10. Clarene. J.P., Degols, G., Leonetti, J.P., Milhaud, P. & Lebleu, B. (1993) *Anticancer Drug Design*, 8, 81.

11. Boussif, O., Lezoulac'h, F., Zanta, M.A., Mergny, M.D., Scherman, E., Demeneix, B. & Behr (1995) *Proc. Natl. Acad. Sci. USA* 92, 7297.

12. Zatloukal, K., Wagner, E. & Cotton, M. (1992) *Proc. Natl. Acad. Sci. USA* 600, 136.

13. Bongartz, J.P., Aubertin, A.M., Milhaud, P.G. & Lebleu B., (1994) *Nucleic Acids Res.* 22, 4681.

14. Derossi, D., Jolit, A.H., Chassaing, G.& Prochiantz, A., (1994) *J. Biol. Chem.* 269, 10444.

15. Citro, G., Perrotti, D., Cucco, C., D'Agnano, I., Sacchi, A., Zupi, G. & Calabretta, B. (1992) *Proc. Natl. Acad. Sci. USA* 89, 7031.

16. Ink, N.H., Beekman, J.M., Kessler, D.J. Murphy, M., Jayaraman, K., Zendegui, J.G., Hogan, M.F., O'Malley, B.W. & Tsai, M.J. (1993) *Nucleic Acids Res.* 21. 2789.

17. Bennett, C.F., Chiang, M.Y., Chan, H., Shoemaker, J.E. & Mirabelli, C.K. (1992) *Mol. Pharmacol.* 41, 1023.

18. Lewis, J.G., et al. (1996) *Proc. Natl. Acad. Sci. USA* 93, 3177.

19. Xu, *et al.* (1998) *Proc. Natl. Acad. Sci. USA* 95, 6642-6646.

CLAIMS:

1. A method for treating metastatic tumor cells of a subject comprising administrating to said subject an antisense molecule, said antisense molecule comprising a nucleotide sequence which is complementary to an RNA sequence of a protease activated receptor (PAR) protein.

2. A method according to claim 1 wherein said PAR protein is a thrombin receptor.

3. A method according to claim 1 wherein said PAR protein is selected from the group consisting of PAR-2, PAR-3 and PAR-4.

4. A method according to claim 1 wherein said tumor cell is of epithelial tissue origin.

5. A method according to claim 4 wherein said epithelial tissue is selected from the group consisting of breast, esophagus, kidney, prostate, ovary, melanoma and bladder.

6. A method according to claim 1 wherein said antisense molecule has the sequence appearing in Fig. 2.

7. A method for treating metastatic tumor cells of a subject comprising administrating to said subject an antibody molecule, said antibody molecule being capable of binding to a protease activated receptor (PAR) protein.

8. A method according to claim 7 wherein said antibody binds an extracellular epitope of said PAR protein.

9. An antisense molecule comprising a nucleotide sequence which is complementary to an RNA sequence of a protease activated receptor (PAR) protein.

10. A pharmaceutical composition comprising an active factor and a pharmaceutically acceptable carrier, said active factor being an antisense molecule comprising a nucleotide sequence which is complementary to an RNA sequence of a protease activated receptor (PAR) protein.

SUBSTITUTE SHEET (RULE 26)

11. A pharmaceutical composition according to claim 10 for the treatment of metastatic tumor cells.

12. A pharmaceutical composition according to claim 11 wherein said PAR protein is a thrombin receptor.

13. A pharmaceutical composition according to claim 11 wherein said PAR protein is selected from the group consisting of PAR-2, PAR-3 and PAR-4.

14. A pharmaceutical composition according to claim 11 wherein said tumor cell is of epithelial tissue origin.

15. A pharmaceutical composition according to claim 14 wherein said epithelial tissue is selected from the group consisting of breast, esophagus, kidney, prostate, ovary, melanoma and bladder.

16. A pharmaceutical composition according to claim 10 wherein said antisense molecule has the sequence appearing in Fig. 2.

17. A method for the treatment of disorders involving the implantation of a placenta in a female subject comprising administrating to said subject an antisense molecule, said antisense molecule comprising a nucleotide sequence which is complementary to an RNA sequence of a protease activated receptor (PAR) protein.

18. A method according to claim 18 wherein said antisense molecule is administered to a trophoblast cell.

19. A pharmaceutical composition according to claim 10 for the treatment of disorders involving the implantation of a placenta in a female subject.

09/744679 PCT/IL99/00079

SUBSTITUTE SHEET (RULE 26)

```
   1 gcgcccgcgc gaccgcgcgc cccagtcccg ccccgccccg ctaaccgccc cagacacagc
  61 gctcgccgag ggtcgcttgg accctgatct tacccgtggg caccctgcgc tctgcctgcc
 121 gcgaagaccg gctccccgac ccgcagaagt caggagagag ggtgaagcgg agcagcccga
 181 ggcggggcag cctcccggag cagcgccgcg cagagcccgg gacaatgggg ccgcggcggc
 241 tgctgctggt ggccgcctgc ttcagtctgt gcggcccgct gttgtctgcc cgcacccggg
 301 cccgcaggcc agaatcaaaa gcaacaaatg ccaccttaga tccccggtca tttcttctca
 361 ggaaccccaa tgataaatat gaaccatttt gggaggatga ggagaaaaat gaaagtgggt
 421 taactgaata cagattagtc tccatcaata aaagcagtcc tcttcaaaaa caacttcctg
 481 cattcatctc agaagatgcc tccggatatt tgaccagctc ctggctgaca ctctttgtcc
 541 catctgtgta caccggagtg tttgtagtca gcctcccact aaacatcatg gccatcgttg
 601 tgttcatcct gaaaatgaag gtcaagaagc cggcggtggt gtacatgctg cacctggcca
 661 cggcagatgt gctgtttgtg tctgtgctcc cctttaagat cagctattac ttttccggca
 721 gtgattggca gtttgggtct gaattgtgtc gcttcgtcac tgcagcattt tactgtaaca
 781 tgtacgcctc tatcttgctc atgacagtca taagcattga ccggtttctg gctgtggtgt
 841 atcccatgca gtccctctcc tggcgtactc tgggaagggc ttccttcact tgtctggcca
 901 tctgggcttt ggccatcgca ggggtagtgc ctctcgtcct caaggagcaa accatccagg
 961 tgcccgggct caacatcact acctgtcatg atgtgctcaa tgaaaccctg ctcgaaggct
1021 actatgccta ctacttctca gccttctctg ctgtcttctt ttttgtgccg ctgatcattt
1081 ccacggtctg ttatgtgtct atcattcgat gtcttagctc ttccgcagtt gccaaccgca
1141 gcaagaagtc ccgggctttg ttcctgtcag ctgctgtttt ctgcatcttc atcatttgct
1201 tcggacccac aaacgtcctc ctgattgcgc attactcatt cctttctcac acttccacca
1261 cagaggctgc ctactttgcc tacctcctct gtgtctgtgt cagcagcata agctcgtgca
1321 tcgaccccct aatttactat tacgcttcct ctgagtgcca gaggtacgtc tacagtatct
```

Fig. 1a

PCT/IL99/00079

09/744679

SUBSTITUTE SHEET (RULE 26)

```
1381 tatgctgcaa agaaagttcc gatcccagca gttataacag cagtgggcag ttgatggcaa
1441 gtaaaatgga tacctgctct agtaacctga ataacagcat atacaaaaag ctgttaactt
1501 aggaaaaggg actgctggga ggttaaaaag aaaagtttat aaaagtgaat aacctgagga
1561 ttctattagt ccccacccaa actttattga ttcacctcct aaaacaacag atgtacgact
1621 tgcatacctg ctttttatgg gagctgtcaa gcatgtattt ttgtcaatta ccagaaagat
1681 aacaggacga gatgacggtg ttattccaag ggaatattgc caatgctaca gtaataaatg
1741 aatgtcactt ctggatatag ctaggtgaca tatacatact tacatgtgtg tatatgtaga
1801 tgtatgcaca cacatatatt atttgcagtg cagtatagaa taggcacttt aaaacactct
1861 ttccccgcac cccagcaatt atgaaaataa tctctgattc cctgatttaa tatgcaaagt
1921 ctaggttggt agagtttagc cctgaacatt tcatggtgtt catcaacagt gagagactcc
1981 atagtttggg cttgtaccac ttttgcaaat aagtgtattt tgaaattgtt tgacggcaag
2041 gtttaagtta ttaagaggta agacttagta ctatctgtgc gtagaagttc tagtgttttc
2101 aattttaaac atatccaagt ttgaattcct aaaattatgg aaacagatga aaagcctctg
2161 ttttgatatg ggtagtattt tttacatttt acacactgta cacataagcc aaaactgagc
2221 ataagtcctc tagtgaatgt aggctggctt tcagagtagg ctattcctga gagctgcatg
2281 tgtccgcccc cgatggagga ctccaggcag cagacacatg ccagggccat gtcagacaca
2341 gattggccag aaaccttcct gctgagcctc acagcagtga gactggggcc actacatttg
2401 ctccatcctc ctgggattgg ctgtgaactg atcatgttta tgagaaactg gcaaagcaga
2461 atgtgatatc ctaggaggta atgaccatga aagacttctc tacccatctt aaaaacaacg
2521 aaagaaggca tggacttctg gatgcccatc cactgggtgt aaacacatct agtagttgtt
2581 ctgaaatgtc agttctgata tggaagcacc cattatgcgc tgtggccact ccaataggtg
2641 ctgagtgtac agagtggaat aagacagaga cctgccctca agagcaaagt agatcatgca
```

Fig. 1a (Cont.)

```
2701 tagagtgtga tgtatgtgta ataaatatgt ttcacacaaa caaggcctgt cagctaaaga
2761 agtttgaaca tttgggttac tatttcttgt ggttataact taatgaaaac aatgcagtac
2821 aggacatata ttttttaaaa taagtctgat ttaattgggc actatttatt tacaaatgtt
2881 ttgctcaata gattgctcaa atcaggtttt cttttaagaa tcaatcatgt cagtctgctt
2941 agaaataaca gaagaaaata gaattgacat tgaaatctag gaaaattatt ctataatttc
3001 catttactta agacttaatg agactttaaa agcatttttt aacctcctaa gtatcaagta
3061 tagaaaatct tcatggaatt cacaaagtaa tttggaaatt aggttgaaac atatctctta
3121 tcttacgaaa aaatggtagc attttaaaca aaatagaaag ttgcaaggca aatgtttatt
3181 taaaagagca ggccaggcgc ggtggctcac gcctgtaatc ccagcacttt gggaggctga
3241 ggcgggtgga tcacgaggtc aggagatcga gaccatcctg gctaacacgg tgaaacccgt
3301 ctctactaaa aatgcaaaaa aaattagccg ggcgtggtgg caggcacctg tagtcccagc
3361 tactcgggag gctgaggcag gagactggcg tgaacccagg aggcggacct tgtagtgagc
3421 cgagatcgcg ccactgtgct ccagcctggg caacagagca agactccatc tcaaaaaaaa
```

Fig. 1a (Cont.)

09/744679
PCT/IL99/00079

SUBSTITUTE SHEET (RULE 26)

MGPRRLLLVAACFSLCGPLLSARTRARRPESKATNATLD
PRSFLLRNPNDKYEPFWEDEEKNESGLTEYRLVSINKSS
PLQKQLPAFISEDASGYLTSSWLTLFVPSVYTGVFVVSL
PLNIMAIVVFILKMKVKKPAVVYMLHLATADVLFVSVLPFK
ISYYFSGSDWQFGSELCRFVTAAFYCNMYASILLMTVISI
DRFLAVVYPMQSLSWRTLGRASFTCLAIWALAIAGVVPL
VLKEQTIQVPGLNITTCHDVLNETLLEGYYAYYFSAFSAV
FFFVPLIISTVCYVSIIRCLSSSAVANRSKKSRALFLSAAV
FCIFIICFGPTNVLLIAHYSFLSHTSTTEAAYFAYLLCVCVS
SISSCIDPLIYYYASSECQRYVYSILCCKESSDPSSYNSS
GQLMASKMDTCSSNLNNSIYKKLLTZ

Fig. 1b

SUBSTITUTE SHEET (RULE 26)

5'-CGCCGAGGGTCGCTTGGACCCTGATCTTACCCGTGGGCACCCTGCGCTCTGCCTGCC

GCGAAGACCGGCTCCCCGACCCGCAGAAGTCAGGAGAGAGGGTGAAGCGGAGCAGCCCGA

GGCGGGGCAGCCTCCCGGAGCAGCGCCGCGCAGAGCCCGGGACAATGGGGCCGCGGCGGC

TGCTGCTGGTGGCCGCCTGCTTCAGTCTGTGCGGCCCGCTGTTGTCTGCCCGCACCCGGG

CCCGCAGGCCAGAATCAAAAGCAACAAATGCCACCTTAGATCCCCGGTCATTTCTTCTCA

GGAACCCCAATGATAAATATGAACCATTTTGGGAGGATGAGGAGAAAAATGAAAGTGGGT

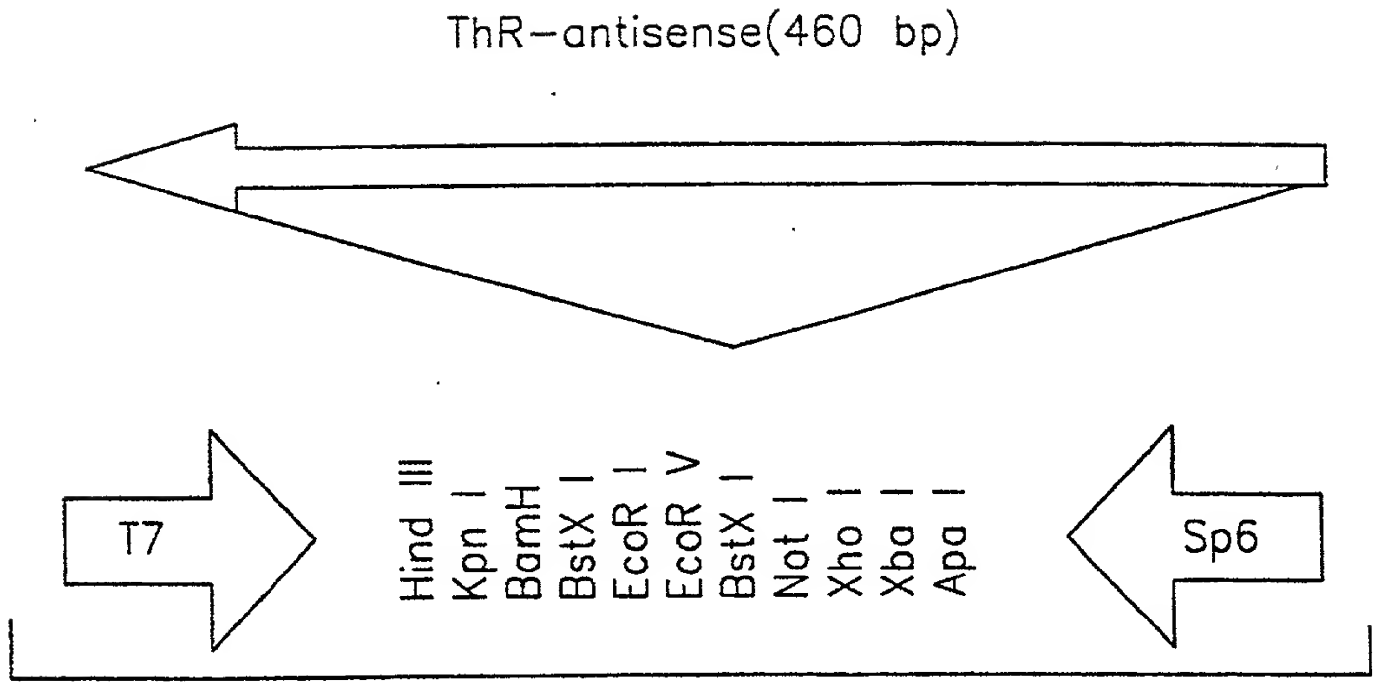
TAACTGAATACAGATTAGTCTCCATCAATAAAAGCAGTCCTCTTCAAAAACAACTTCCTG

CATTCATCTCAGAAGATGCCTCCGGATATTTGACCAGCTCCTGGCTGACACTCTTTGTCC

CATCTGTGTACACCGGAGTGTTTGTAGTCAGCCTCCCACTAAACATCATGGCCATCGTTG

TGTTCATCCTG-3'

Fig. 2


ThR-antisense(460 bp)
T7
Hind III
Kpn I
BamH
BstX I
EcoR I
EcoR V
BstX I
Not I
Xho I
Xba I
Apa I
Sp6

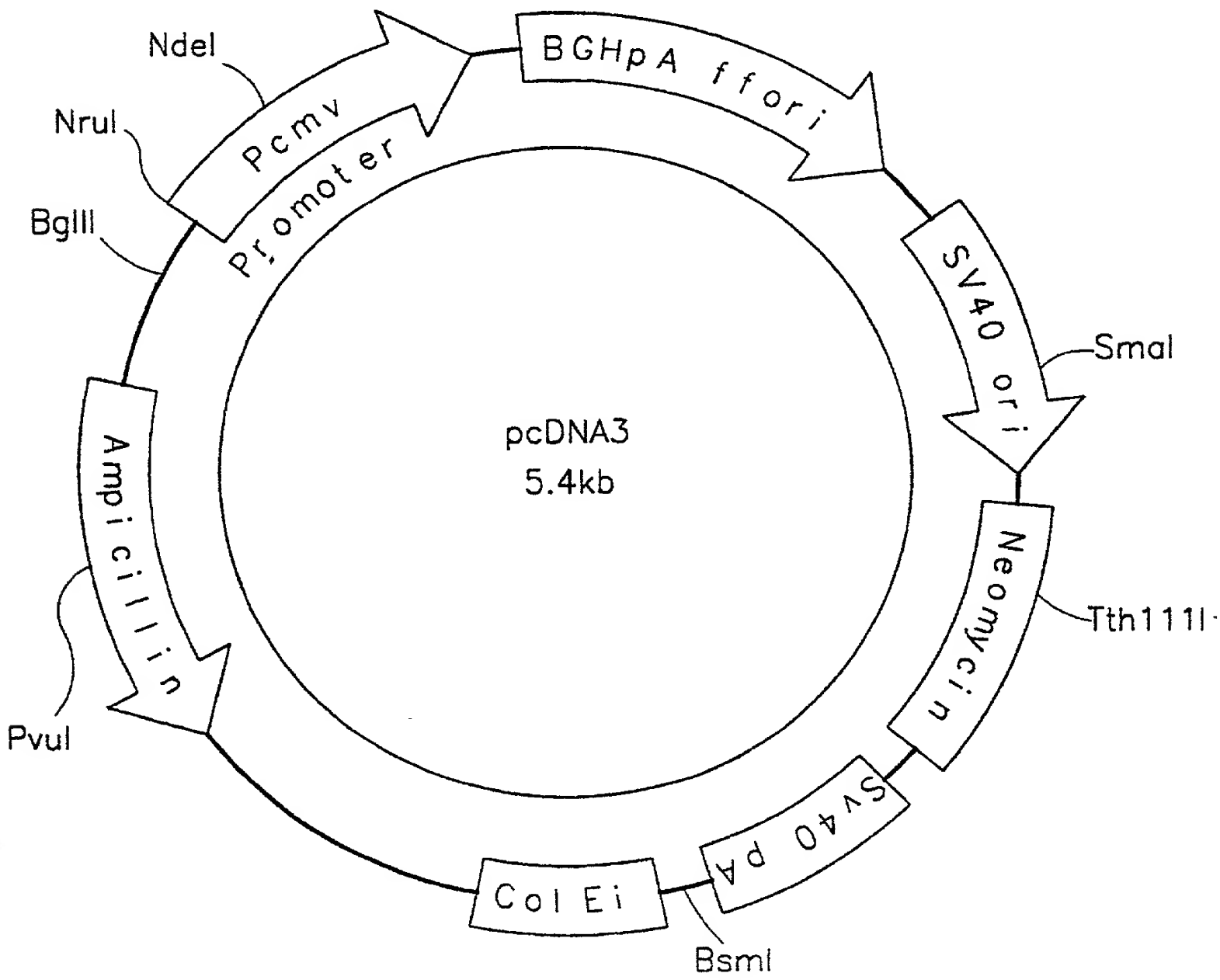

Ndel
Nrul
BglII
PvuI
Pcmv Promoter
BGHpA
f1 ori
SV40 ori
SmaI
Neomycin
Tth111I
SV40 pA
Col Ei
BsmI
Ampicillin
pcDNA3
5.4kb

FIG.3

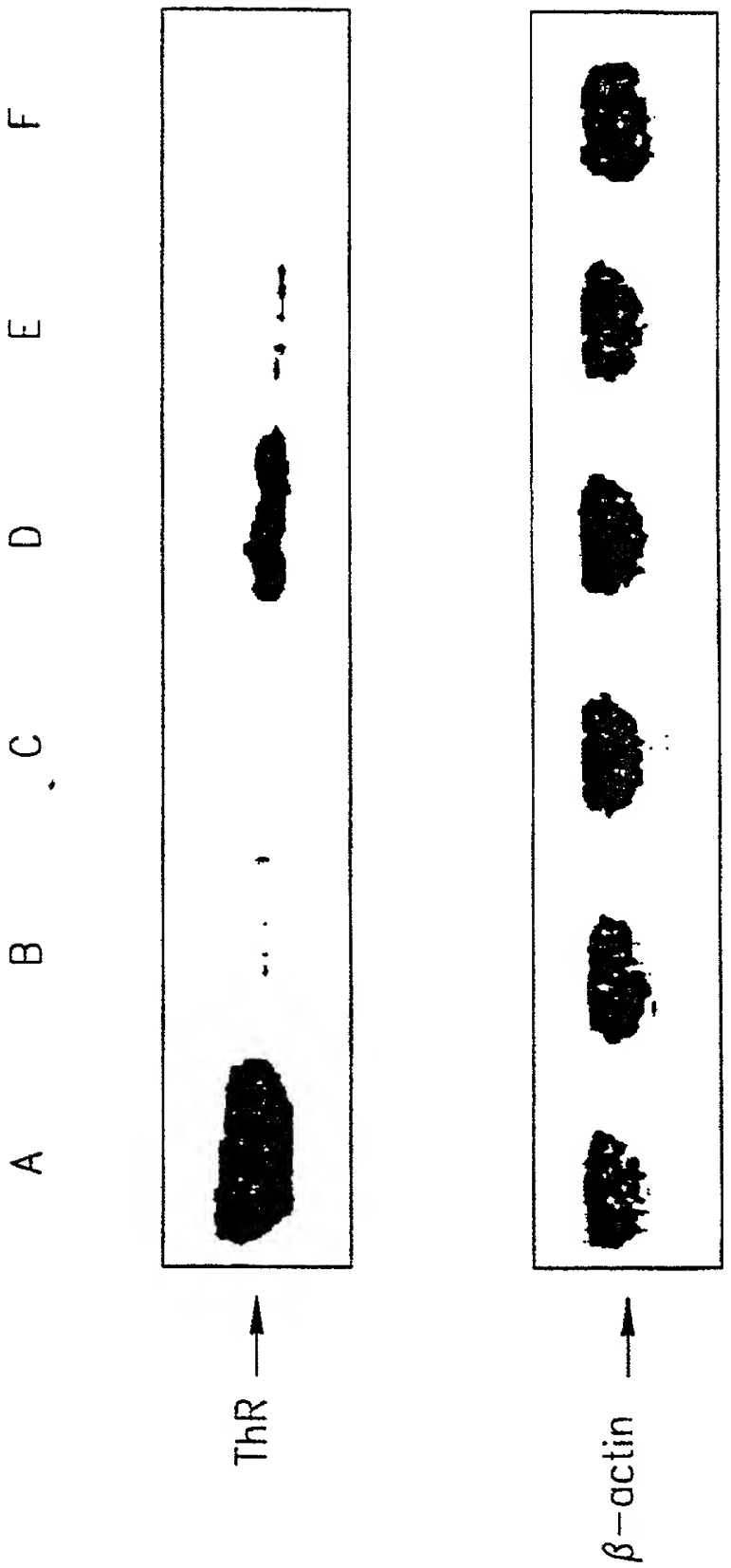
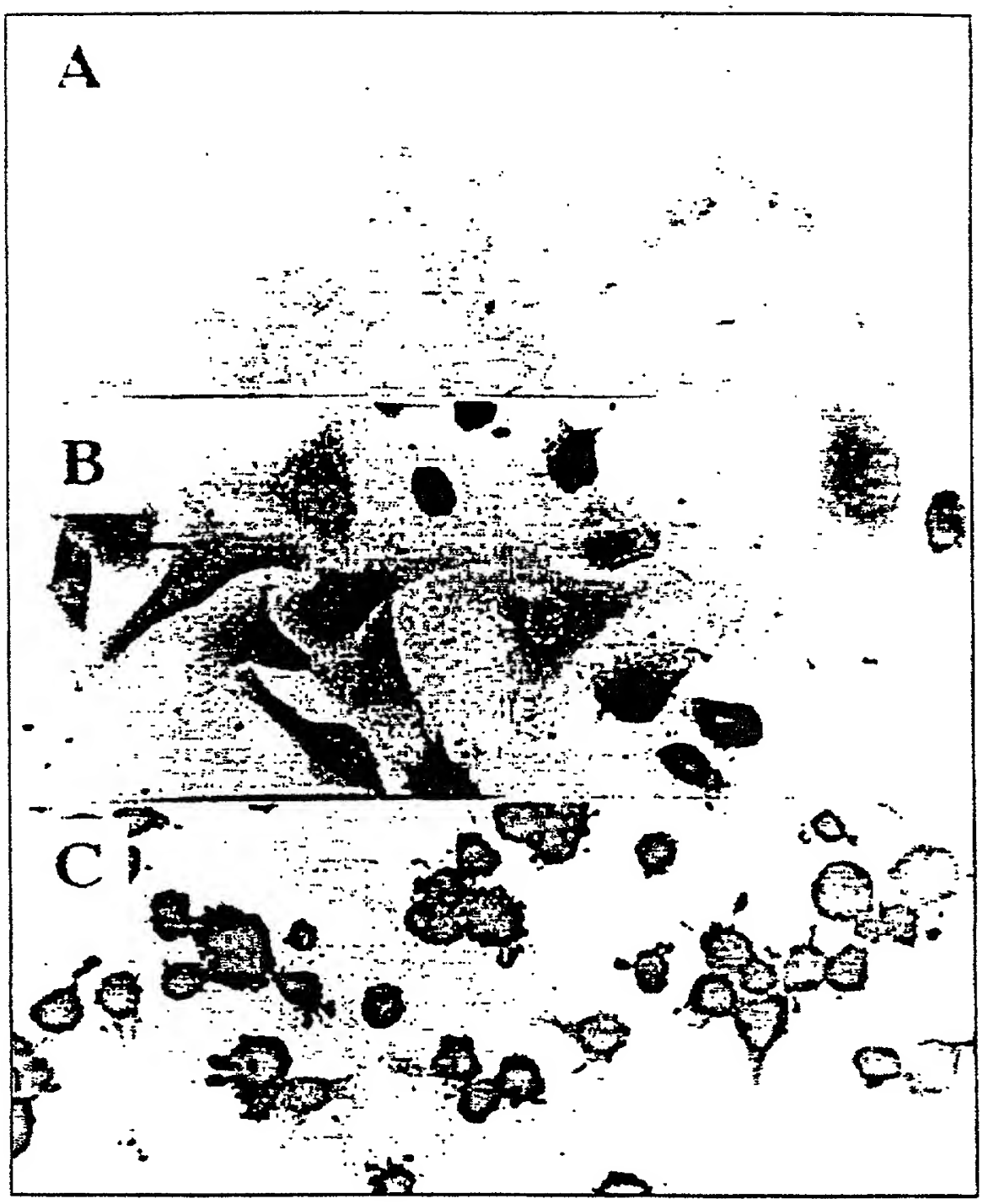


FIG.4



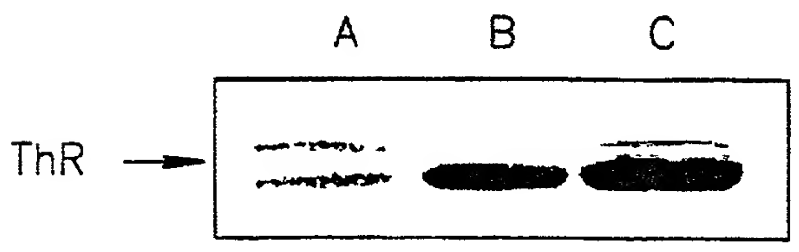


FIG.5

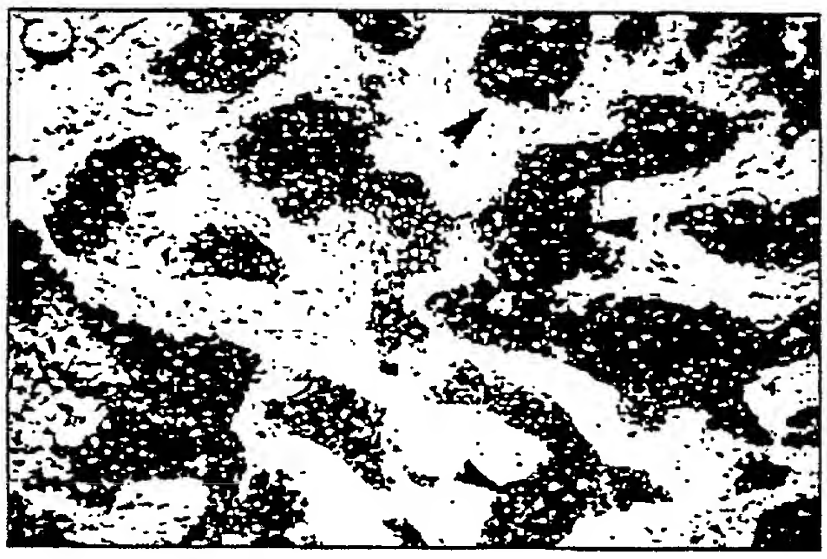


FIG.6C

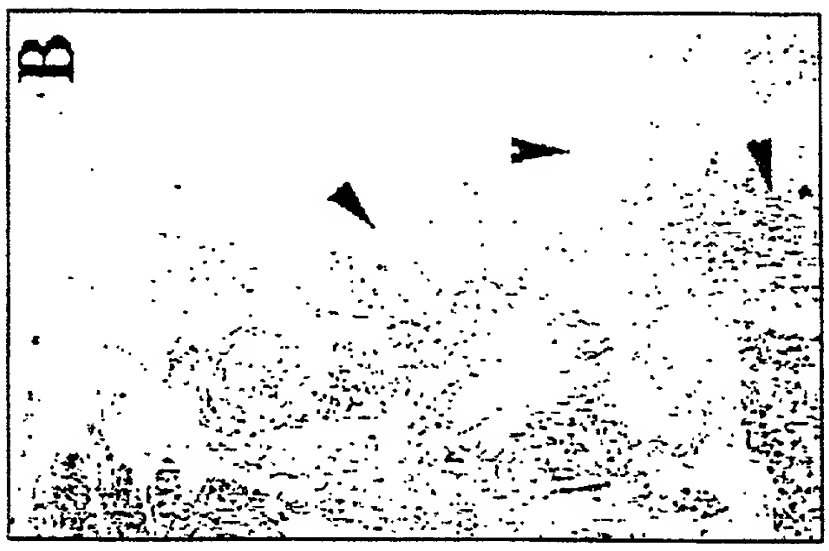


FIG.6B

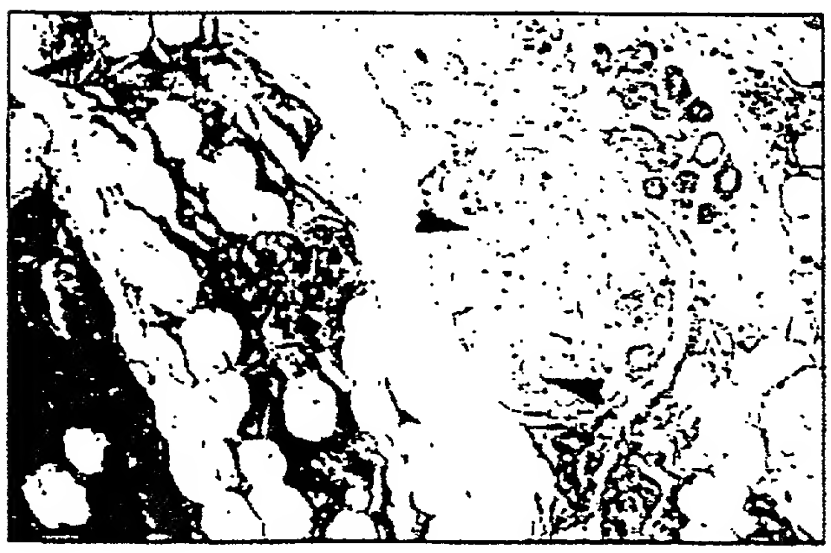


FIG.6A

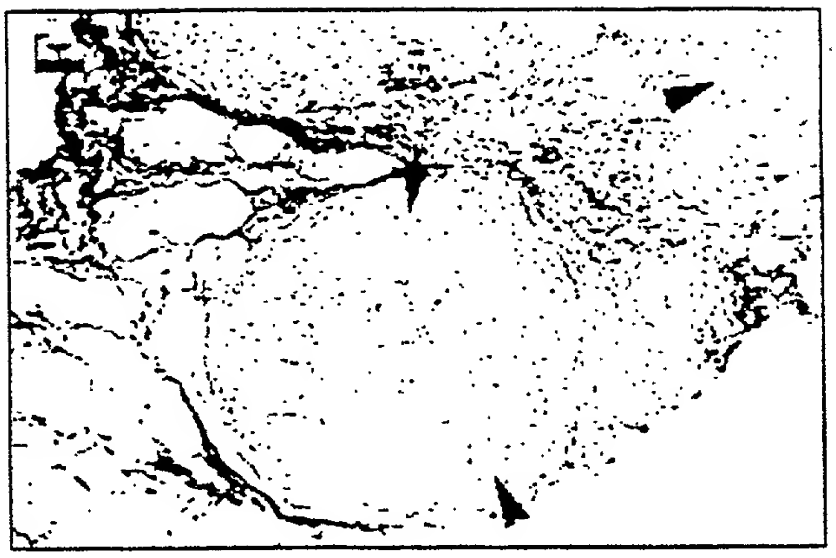


FIG.6F

FIG.6E

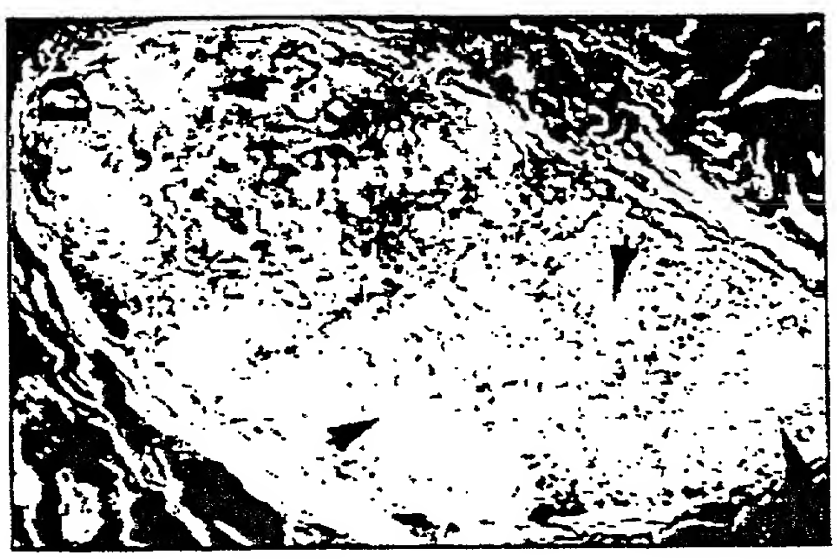


FIG.6D

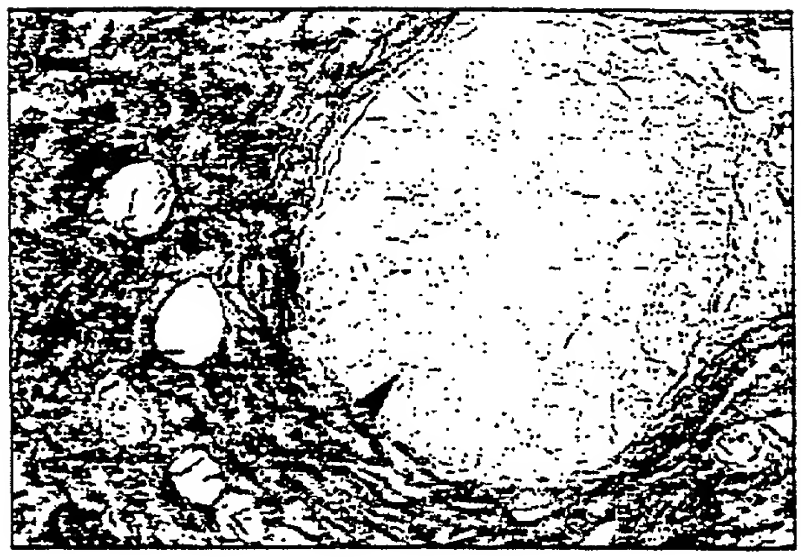


FIG.6I

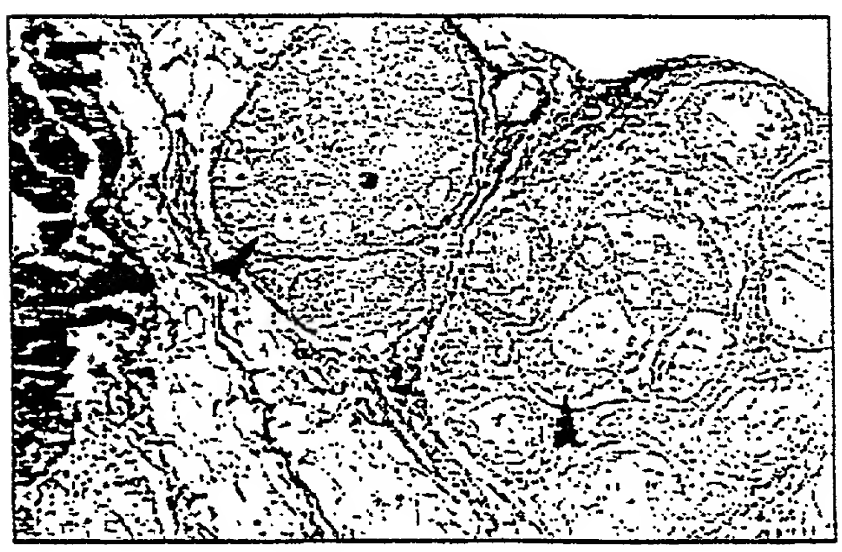


FIG.6H

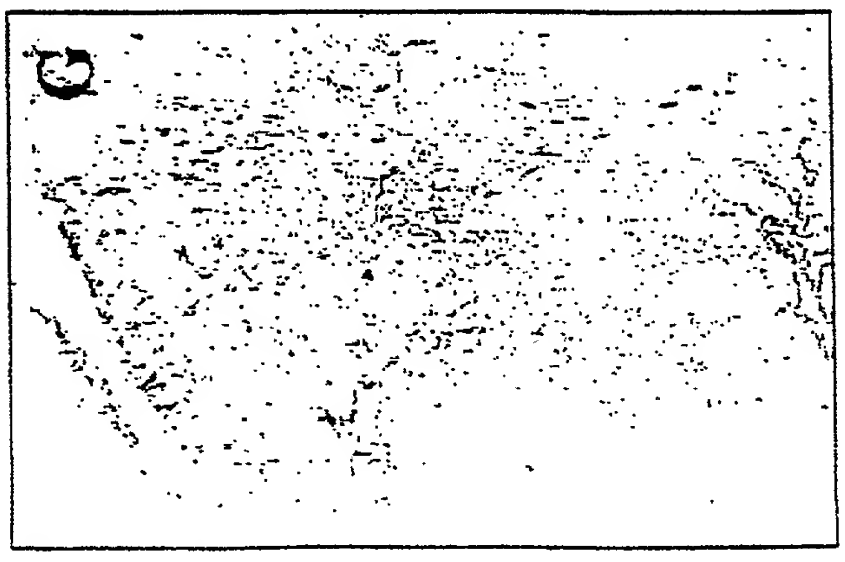


FIG.6G

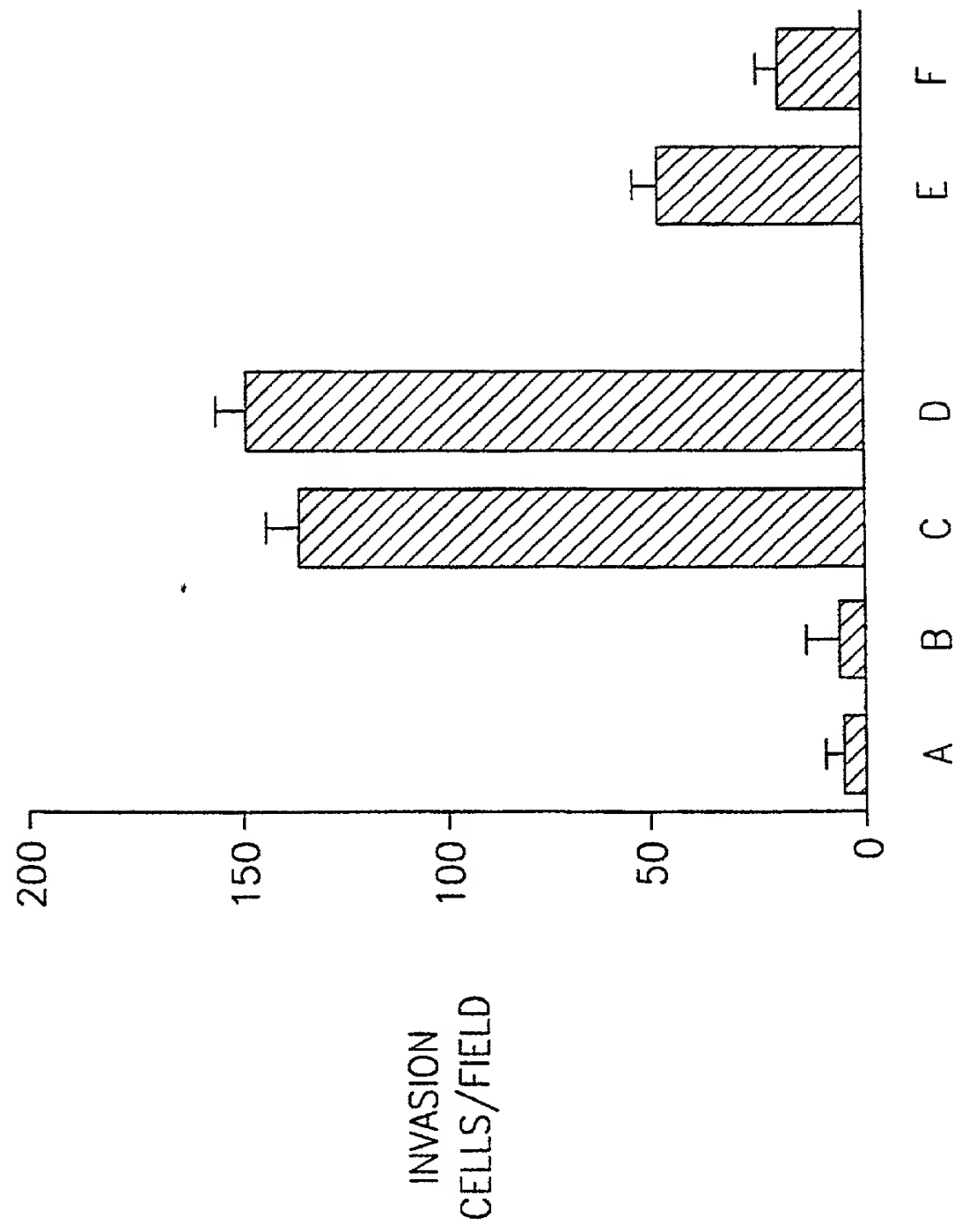


FIG.7

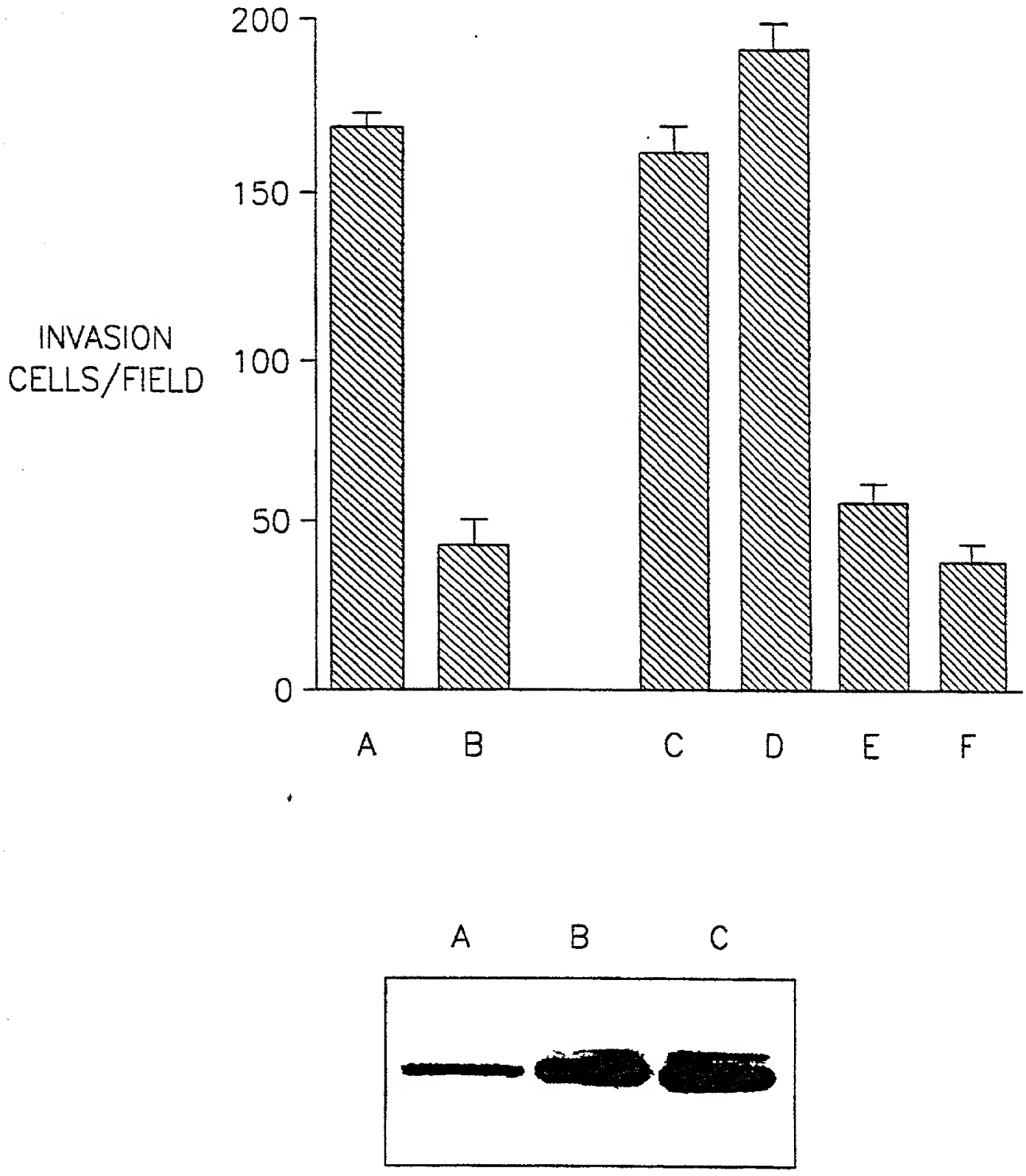


FIG.8

```
   1 aaaatgaata aatgaatgta ctttcatttg aacaaaccag tgttactgct gaaacattta
  61 tttctgtaat gacccttgtc ttcctttctt gtacaggaac caatagatcc tctaaaggaa
 121 gaagccttat tggtaaggtt gatggcacat cccacgtcac tggaaaagga gttacagttg
 181 aaacagtctt ttctgtggat gagttttctg catctgtcct cactggaaaa ctgaccactg
 241 tcttccttcc aattgtctac acaattgtgt ttgtggtggg tttgccaagt aacggcatgg
 301 ccctgtgggt ctttctttttc cgaactaaga agaagcaccc tgctgtgatt tacatggcca
 361 atctggcctt ggctgacctc ctctctgtca tctggttccc cttgaagatt gcctatcaca
 421 tacatggcaa caactggatt tatggggaag ctctttgtaa tgtgcttatt ggctttttct
 481 atggcaacat gtactgttcc attctcttca tgacctgcct cagtgtgcag aggtattggg
 541 tcatcgtgaa ccccatgggg cactccagga agaaggcaaa cattgccatt ggcatctccc
 601 tggcaatatg gctgctgatt ctgctggtca ccatcccttt gtatgtcgtg aagcagacca
 661 tcttcattcc tgccctgaac atcacgacct gtcatgatgt tttgcctgag cagctcttgg
 721 tgggagacat gttcaattac ttcctctctc tggccattgg ggtctttctg ttcccagcct
 781 tcctcacagc ctctgcctat gtgctgatga tcagaatgct gcgatcttct gccatggatg
 841 aaaactcaga gaagaaaagg aagagggcca tcaaactcat tgtcactgtc ctggccatgt
 901 acctgatctg cttcactcct agtaaccttc tgcttgtggt gcattatttt ctgattaaga
 961 gccagggcca gagccatgtc tatgccctgt acattgtagc cctctgcctc tctaccctta
1021 acagctgcat cgaccccttt gtctattact ttgtttcaca tgatttcagg gatcatgcaa
1081 agaacgctct cctttgccga agtgtccgca ctgtaaagca gatgcaagta tccctcacct
1141 caaagaaaca ctccaggaaa tccagctctt actcttcaag ttcaaccact gttaagacct
1201 cctattgagt tttccaggtc ctcagatggg aattgcacag taggatgtgg aacctgttta
1261 atgttatgag gacgtgtctg ttatttcct
```

Fig. 9

```
   1 cggcacgagc aaggacgagt ccctgcccac acagtccagg ctggcagagt tctcagcttt
  61 ccacttgctg ctcatacatg gagctgaggg gaatctaccc tgtgacttcg tatacttaac
 121 aacatcctgt agccgggtct caggacatca agatgaaaat ccttatcttg gttgcagctg
 181 ggctgctgtt tctgccagtc actgtttgcc aaagtggcat aaatgtttca gacaactcag
 241 caaagccaac cttaactatt aagagtttta atggggctcc ccaaaatacc tttgaagaat
 301 tcccactttc tgacatagag ggctggacag gagccaccac aactataaaa gcggagtgtc
 361 ccgaggacag tatttcaact ctccacgtga ataatgctac cataggatac ctgagaagtt
 421 ccttaagtac ccaagtgata cctgccatct atatcctgct gtttgtggtt ggtgtaccat
 481 ccaacatcgt gaccctgtgg aaactctcct taaggaccaa atccatcagt ctggtcatct
 541 ttcacaccaa cctggccatc gcagatctcc ttttctgtgt cacactgcca tttaagatcg
 601 cctaccatct caatggcaac aactgggtat ttggcgaggt catgtgccgg atcaccacgg
 661 tcgttttcta cggcaacatg tactgcgcta tcctgatcct cacttgcatg ggcatcaacc
 721 gctacctggc cacggctcac cctttcacat accagaagct gcccaaacgc agcttctcct
 781 tgctcatgtg tggcatagtg tgggtcatgg ttttcttata catgctgccc tttgtcatcc
 841 tgaagcagga gtaccacctc gtccactcag agatcaccac ctgccacgat gtcgtcgacg
 901 cgtgcgagtc cccatcatcc ttccgattct actacttcgt ctccttagca ttcttttgcg

 961 tcctcatccc gtttgtgatc atcatcttct gttacacgac tctcatccac aaacttaaat
1021 caaaggatcg gatatggctg ggctacatca aggccgtcct cctcatcctt gtgattttca
1081 caatttgctt tgcccccacc aacatcatac tcgtaatcca ccatgccaac tactactacc
1141 acaataccga cagcttgtac tttatgtatc ttattgctct gtgcctgggg agcctgaata
1201 gctgcctaga tccattcctt tactttgtca tgtcgaaagt tgtagatcag cttaatcctt
1261 agtcggcaat ggcaagacca ctttagagac caaggagaga tatctgggaa gacatacatg
1321 cttggctgac ttatgcatgg caccaacagc tcaatttttа attttaattt aattttatct
1381 ttttgagaca gaacctcact gtgtagtcct ggctggcctg gctgattctc tatttagacc
1441 aggttagcct tgaactcaca gagatctgcc tgcttctgcc tcccaagtgc tgggttcaac
1501 caggtctggc aagcgctcca tttgtcagct cctctgcaac agtgctttat acttccaatg
1561 tgaagtcatg aggattaaaa gaatactttg tatttagaga gtgtcttaat tgtaagcaac
1621 attagcagta ttccgatggc aatggacgca tttctatttg ccttcctgat tctccaagct
1681 atacatgtcc ccttccctcc tgtgagcggt gtcggcagac tgggaggttg tcactacccg
1741 tggttctccc tcctcctcaa gcaggtatcc tgtctttgtt gtgtctctca ctggggtccc
1801 gtgttcatgc cacctctgtc caaagatggc agcctcactg ccagccgagc ctcatttcca
1861 cagattgggt taaccttccc tgaacagttt ctgaatacat catgcaatac ttcacttgac
1921 cctgcagtgc tcctgaaatg acaagcccct gcatggcttt cctggtggag ctgtgcctgc
1981 gattccacac accttcacac cgactgtctt ccctcccatc cctgtcgctc cactgaaacc
2041 ccttctagca tctttgcttt agcctgtcca gcttttcaag tcgttaaagg cccattcttc
2101 ccttattctc aaactaaaaa catcattttc tttatctgga tttgtaaagc actttcttat
2161 ctttaaggca cttctagaca aacaagcctg ctaagctcat ttgcatatag atattacttc
2221 cataaggaat taagttctct gtgtggtcag ctctgtatcc ctgcaagttt catacctgta
2281 gattcaacca accacagacc caaaacactt gaaaaacctt gtatgagctg aaaacgaaga
2341 gttctatttt tcattactcc ntaaataata ntgataataa ataataataa ataaaaaaaa
2401 aaaaaaaaa
```

Fig 10

SUBSTITUTE SHEET (RULE 26)

09/744679

1 ctcccacggg ctggctggca agcggccctg gtgggtctgc gggggcaggg gcagccttcc
61 tggtttatct ccaccggcgc gatctgctcg tccgcctcgg ctccagaagc tggggctcag
121 ggtccggcga ggcaggaagc ctgaggccac agcccagagc agcctgagtg cagtcatgtg
181 ggggcgactg ctcctgtggc ccctggtgct ggggttcagc ctgtctggcg gcacccagac
241 ccccagcgtc tacgacgaga gcgggagcac cggaggtggt gatgacagca cgccctcaat
301 cctgcctgcc ccccgcggct acccaggcca agtctgtgcc aatgacagtg acaccctgga
361 gctcccggac agctcacggg cactgcttct gggctgggtg cccaccaggc tggtgcccgc
421 cctctatggg ctggtcctgg tggtggggct gccggccaat gggctggcgc tgtgggtgct
481 ggccacgcag gcacctcggc tgccctccac catgctgctg atgaacctcg cgactgctga
541 cctcctgctg gccctggcgc tgcccccgcg gatcgcctac cacctgcgtg gccagcgctg
601 gcccttcggg gaggccgcct gccgcctggc cacggccgca ctctatggtc acatgtatgg
661 ctcagtgctg ctgctggccg ccgtcagcct ggatcgctac ctggccctgg tgcacccgct
721 gcgggcccgc gccctgcgtg gccggcgcct ggcccttgga ctctgcatgg ctgcttggct
781 catggcggcc gccctggcac tgcccctgac actgcagcgg cagaccttcc ggctggcgcg
841 ctccgatcgc gtgctctgcc atgacgcgct gcccctggac gcacaggcct cccactggca
901 accggccttc acctgcctgg cgctgttggg ctgtttcctg cccctgctgg ccatgctgct
961 gtgctacggg gccaccctgc acacgctggc ggccagcggc cggcgctacg gccacgcgct
1021 gaggctgacc gcagtggtgc tggcctccgc cgtggccttc ttcgtgccca gcaacctgct
1081 gctgctgctg cattactcgg acccgagccc cagcgcctgg ggcaacctct atggtgccta
1141 cgtgcccagc ctggcgctga gcaccctcaa cagctgcgtg gatcccttca tctactacta

Fig. 11a

09/744,679

PCT/IL99/00079

SUBSTITUTE SHEET (RULE 26)

1201 cgtgtcggcc gagttcaggg acaaggtgcg ggcagggctc ttccaacggt cgccggggga
1261 caccgtggcc tccaaggcct ctgcggaagg gggcagccgg ggcatgggca cccactcctc
1321 tttgctccag tgacacaaag tggggaaggc tgtactgggt cgaacagggt cccttccccc
1381 acttcacgtc cttcctggga cctcagaatg tgaccttatt tggaaatagg gttgttacaa
1441 ctgtcactag cggaggtcac tttggagaag ggtgggcctt acatccagtg tgggtggtgt
1501 cctcataaga taaggagagg ccaggcctgg tggctcacgc ctgtaatccc agcactttaa
1561 gaggccaagg cggatggatc acttgagccc aggagttcaa caccagcctg agcaacatgg
1621 taaaacccca tctctaccaa aaatacaaaa attagctggg cttggtggct ggcgcctgta
1681 atcccagcta ctcaggagac tgaggcagaa ggatcgcttg aacctgggag gcagaggttg
1741 cagtgagccg agattgcgcc actggactcc agcctgcgtg acagagagcc tgtctctaaa
1801 ttaattaatt aattaattta attcaatttt aaaaagacga aaagtgacgg ccaggtgcag
1861 tggctcacgc ctataatctc agcactctgg gaggccaaga tggaggattg cttgaagcca
1921 ggagtttggg accagcctgg gcaacatagg gggatcccat ctctacacac aaaaaaattt
1981 tttaatgaac caggcattgt ggcatgcgcc tatagtccca gccactcaag aggcacaggc
2041 gggaggatca cttgagcctg ggaggttgtg gttgcagtga gctatgattg taccactgca
2101 ctccagcctg ggcaacagag caagaccttg tctcaaaaat aaacaaacta aaattaaaaa
2161 aagaagacga gagatagtgg gtgtggtggc tcacacctgc aatcccagca ctttggaagg
2221 ccgaggtggg cagatcatct gaggccagga gttcaagacc agcctggcta acatggtgaa

Fig. 11a (Cont.)

SUBSTITUTE SHEET (RULE 26)

09/744679
PCT/IL99/00079

2281 atcctatctc taccaaaaat acaaaaatta gccaggcgtg gtggtgggca cctgtactgg
2341 ggaggtgccc acccagctac tggggaggct gagtcaggag aatcgcttga acctgggagg
2401 cggaggttgc ggtcagctga gatggtgcca ctgcactcca gcctgggcga aagagcgact
2461 ctgtctccaa aaaaaagaga agaggagagg acacagagac acacagagaa gaaagccatg
2521 tggcggcaga ggcagagatg ggagtgatgc ggacggacac aaactaaggg atgccacgat
2581 gccaagcaca gccaacagcc accagcagcc aggagacagg cctgggacgg gctctccctc
2641 acagcctcca gagggaacca gccctgccac caccttgacc ctggacttct ggcctgcaga
2701 actgtgagac aataaactct cattgtttta agctgcctgg catgtggcac tttgtcaggg
2761 cagcccagga atctgaaaca ggatcaaact ctgcttcctg ggccctgcca gcatctctgg
2821 ctcggctttc tgggctggat gcagcccacg acgcactggt gtctgagatg gggctggagc
2881 tggggctggg gctgcattcc ctggagactc actgcaagtt cctgcccagg aggctgaggg
2941 caccccatcc tcagtgccca atgctgtggc cccaccaggc ccagagcctg gttggccatt
3001 ctcatgccca ccagcttctg gctttgggat gtctcttgag caaccagaat agcacccccа
3061 actctgctcc ccaaaaccca tcactagcac ggctcagcct cctgctatcc cctgactgct
3121 ggggaccctc gccttccctc ctctcacctg caggctgatc cttcttttca ctttctgtca
3181 atgtcaccag ggataaggtg ggacaatggg gggtgggggt ggacagtgtg tgctgggggg
3241 ttcgggtgct gcagacctgg aactcccttc tgccaggatg ttggcagccg gttgtaagcc
3301 ttgcacggga cagaccacac ccaccgcaac ctcatcccct cagcactaac cacatccact

Fig. 11a (Cont.)

SUBSTITUTE SHEET (RULE 26)

3361 ctcaaccccg tcccttcgc actgaccaca cccaccccgt tcggccccgc cccccgcact
3421 gaacactccc gccctcaacc ccgcaccctc cgcactcacc tccccctcgc cgctcgaccc
3481 cgccctcacc acactgacca ccctcaaccc attgcgccca gtccccacca cagtgaccac
3541 accctcactg gctcggccct gcccccagta tactgaccat tccccagcca cttcccttcc
3601 gcacttacca ctccccccagc cacgcccctc cccgctgacc gctcctccag ccccgcctcc
3661 cccgtacagg cagagcgccc gcccacctct atgctgcgtt ctcctgactt tacgttggcc
3721 cctcctctgc caagccccca ggggagccct ccctggcgtc cgagggtggg agtcggggtg
3781 tggcaggccg cggtgggggg cggcagtggc tccgcgcact cacccgggcc ccgggcaggg
3841 gcgcgctcca cttcgttgca cgcgggtccg gcgcacagtt cccgggcgag tgggctgtgc
3901 gtgctgacgt tgtagaagcg agtggcctcg aaggctacgg gacgagggtg gcgggtgacc
3961 aagtgcaggc gcgacgggtc agggaccggg ccgggccggg ggtgcgggcg cgcgggccta
4021 ccgggttcgt agtagtcgta cacggagact ggcagcgccg acgtcctgcc caccacgcac
4081 tcccggagag cacggaaccg cacgcacgtc aggcaccggc tggggatctg tggggcagcg
4141 gcgggcgcag gctcgacccg ggccaggagg cccggggcgc tgagctcagg cccagaactg
4201 gctgatttca gggataccca ggacgcgtga aacacagaag aaacgtgatc ccatttttctt
4261 tttttctttt actttttcttt tttttttttt ttcctgagac agagtctcgc gctgttgccc
4321 aggctggagt gcagtggcgt gatctcggct cactgcaagc tcggcctcct gggttcaaat
4381 gattctcctg cctcagcctc ccaagtagct gggataacag gcgcccacca ccgcaccctg

Fig. 11a (Cont.)

SUBSTITUTE SHEET (RULE 26)

4441 ctaatttttt gtatttttga tcaagacgga gtttcaccat gttggccagg ctggtctcca
4501 actcctgccc tcaagtgatc cgcctcggtc ccatttttta ttctttgggt ccttccatcc
4561 cactgggaaa acgtctcagg tggcctctga aacaccactc ctttttgtgt gtgtgcacgc
4621 atggctgagc atgtgtgggt gggagtcagc acattcacga tactgtgcaa tcatcacctc
4681 tgtctagtta caggacggtt tctttctccc ccaaagaaac cccatcgcca tcagcactca
4741 ctcccccactc ccccagcccc tggcaaccac aaatctttcc aactctacgg atttgcctgt
4801 tctgggcatt tcatgtcaat ggaatcatgt actctgtgaa aaaaaaaaaa aaaaaaaaaa
4861 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaa

Fig. 11a (Cont.)

SUBSTITUTE SHEET (RULE 26)

MWGRLLLWPLVLGFSLSGGTQTPSVYDESGSTGGGDDSTPSILP

APRGYPGQVCANDSDTLELPDSSRALLLGWVPTRLVPALYGLVLVVGLPANGLALWVL

ATQAPRLPSTMLLMNLATADLLLALALPPRIAYHLRGQRWPFGEAACRLATAALYGHM

YGSVLLLAAVSLDRYLALVHPLRARALRGRRLALGLCMAAWLMAAALALPLTLQRQTF

RLARSDRVLCHDALPLDAQASHWQPAFTCLALLGCFLPLLAMLLCYGATLHTLAASGR

RYGHALRLTAVVLASAVAFFVPSNLLLLLHYSDPSPSAWGNLYGAYVPSLALSTLNSC

VDPFIYYYVSAEFRDKVRAGLFQRSPGDTVASKASAEGGSRGMGTHSSLLQ

Fig. 11b

WO 00/08150

21/24

09/744679

PCT/IL96/00079

FIG.12C

FIG.12B

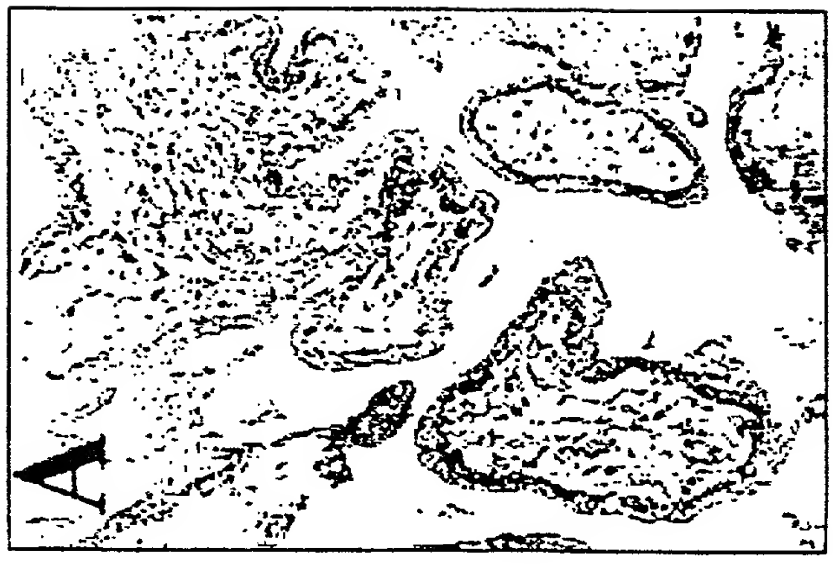


FIG.12A

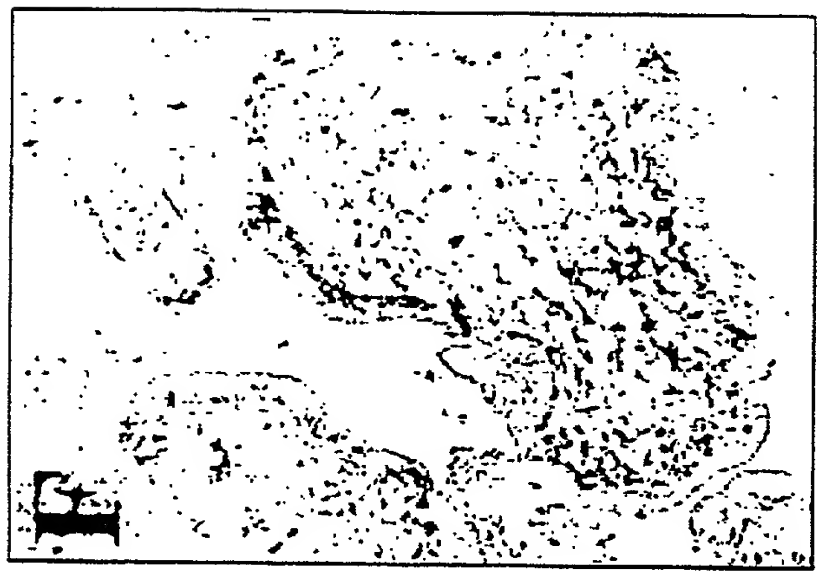


FIG.12F

FIG.12E

FIG.12D

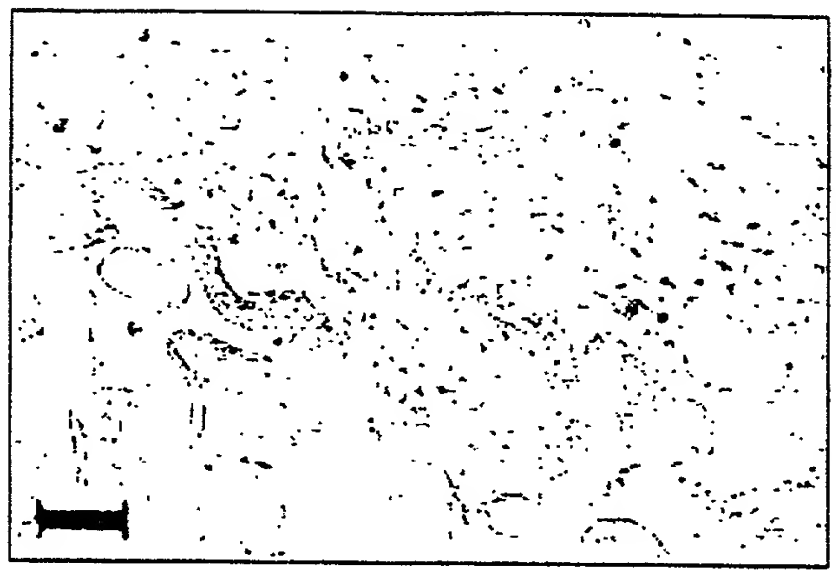


FIG.12I

FIG.12H

FIG.12G

Docket No.: 108366

DECLARATION AND POWER OF ATTORNEY UNDER 35 USC §371(c)(4) FOR PCT APPLICATION FOR UNITED STATES PATENT

As a below named inventor, I hereby declare that:
my residence, post office address and citizenship are as stated below under my name;

I verily believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought, namely the invention entitled: METHOD FOR TREATMENT OF INVASIVE CELLS

described and claimed in international application number PCT/IL99/00079 filed February 5, 1999.

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations §1.56.

Under Title 35, U.S. Code §119, the priority benefits of the following foreign application(s) filed by me or my legal representatives or assigns within one year prior to my international application are hereby claimed:

Israeli Patent Application No. 125698 filed August 7, 1998.

The following application(s) for patent or inventor's certificate on this invention were filed in countries foreign to the United States of America either (a) more than one year prior to my international application, or (b) before the filing date of the above-named foreign priority application(s):

I hereby appoint the following as my attorneys of record with full power of substitution and revocation to prosecute this application and to transact all business in the Patent Office:

James A. Oliff, Reg. No. 27,075; William P. Berridge, Reg. No. 30,024; Kirk M. Hudson, Reg. No. 27,562; Thomas J. Pardini, Reg. No. 30,411; Edward P. Walker, Reg. No. 31,450; Robert A. Millar, Reg. No. 32,771; Mario A. Costantino, Reg. No. 33,565; Stephen J. Rose, Reg. No. 34,463; Joel S. Armstrong, Reg. No. 36,430; Christopher W. Brown, Reg. No. 38,025; and Richard E. Rice, Reg. No. 31,560.

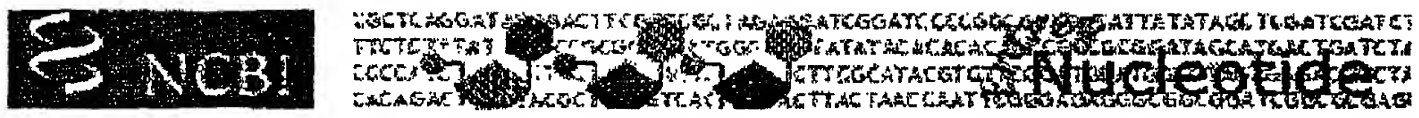
ALL CORRESPONDENCE IN CONNECTION WITH THIS APPLICATION SHOULD BE SENT TO OLIFF & BERRIDGE, PLC, P.O. BOX 19928, ALEXANDRIA, VIRGINIA 22320, TELEPHONE (703) 836-6400.

I hereby declare that I have reviewed and understand the contents of this Declaration, and that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1	*Typewritten Full Name of Sole or First Inventor*	Rachel		BAR-SHAVIT
		Given Name	Middle Initial	Family Name
2	**Inventor's Signature**	Rachel Bar Shavit		
3	**Date of Signature**	March	22	2001
		Month	Day	Year
	Residence:	Ramat-Sharet	Jerusalem	Israel ILX
		City	State or Province	Country
	Citizenship:	Israel		
	Post Office Address: (Insert complete mailing address, including country)	44 Peretz Berenstein Street		
		Ramat-Sharet Jerusalem 96920 Israel		

Note to Inventor: Please sign name on line 2 exactly as it appears in line 1 and insert the actual date of signing on line 3.

IF THERE IS MORE THAN ONE INVENTOR USE PAGE 2 AND PLACE AN "X" HERE ☐
(Discard this page in a sole inventor application)



1: U92971 **Human protease-activated receptor 3 (PAR3) mRNA, complete cds**

PubMed, Protein, Related Sequences, Taxonomy, OMIM, LinkOut

```
LOCUS       HSU92971     1830 bp    mRNA            PRI      16-APR-1997
DEFINITION  Human protease-activated receptor 3 (PAR3) mRNA, complete cds.
ACCESSION   U92971
VERSION     U92971.1  GI:1938374
KEYWORDS    .
SOURCE      human.
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 1830)
  AUTHORS   Ishihara,H., Connolly,A.J., Zeng,D., Kahn,M.L., Zheng,Y.W.,
            Timmons,C., Tram,T. and Coughlin,S.R.
  TITLE     Protease-activated receptor 3 is a second thrombin receptor in
            humans
  JOURNAL   Nature 386 (6624), 502-506 (1997)
  MEDLINE   97242411
REFERENCE   2  (bases 1 to 1830)
  AUTHORS   Ishihara,H., Connolly,A.J., Zeng,D., Kahn,M.L., Zheng,Y.W.,
            Timmons,C., Tram,T. and Coughlin,S.R.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-MAR-1997) CVRI, UCSF, 3rd and Parnassus, San
            Francisco, CA 94143, USA
FEATURES             Location/Qualifiers
     source          1..1830
                     /organism="Homo sapiens"
                     /db_xref="taxon:9606"
     gene            145..1270
                     /gene="PAR3"
     CDS             145..1269
                     /gene="PAR3"
                     /note="thrombin receptor; coagulation protease"
                     /codon_start=1
                     /product="protease-activated receptor 3"
                     /protein_id="AAC51218.1"
                     /db_xref="GI:1938375"
                     /translation="MKALIFAAAGLLLLLPTFCQSGMENDTNNLAKPTLPIKTFRGAP
                     PNSFEEFPFSALEGWTGATITVKIKCPEESASHLHVKNATMGYLTSSLSTKLIPAIYL
                     LVFVVGVPANAVTLWMLFFRTRSICTTVFYTNLAIADFLFCVTLPFKIAYHLNGNNWV
                     FGEVLCRATTVIFYGNMYCSILLLACISINRYLAIVHPFTYRGLPKHTYALVTCGLVW
                     ATVFLYMLPFFILKQEYYLVQPDITTCHDVHNTCESSSPFQLYYFISLAFFGFLIPFV
                     LIIYCYAAIIRTLNAYDHRWLWYVKASLLILVIFTICFAPSNIILIIHHANYYYNNTD
                     GLYFIYLIALCLGSLNSCLDPFLYFLMSKTRNHSTAYLTK"
BASE COUNT      473 a    464 c    337 g    556 t
ORIGIN
        1 cctgcctgca cggcacagga gagcaaactt ctacagacag accaaggctt ccatttgctg
       61 ctgacacatg gaactgaggt gaaattgtgc tccatgattt tacagatttc ataacgttta
      121 agagacggga ctcaggtcat caaaatgaaa gccctcatct ttgcagctgc tggcctcctg
      181 cttctgttgc ccacttttg tcagagtggc atggaaaatg atacaaacaa cttggcaaag
      241 ccaaccttac ccattaagac ctttcgtgga gctcccccaa attcttttga agagttcccc
```

```
 301 ttttctgcct tggaaggctg gacaggagcc acgattactg taaaaattaa gtgccctgaa
 361 gaaagtgctt cacatctcca tgtgaaaaat gctaccatgg ggtacctgac cagctcctta
 421 agtactaaac tgatacctgc catctacctc ctggtgtttg tagttggtgt cccggccaat
 481 gctgtgaccc tgtggatgct tttcttcagg accagatcca tctgtaccac tgtattctac
 541 accaacctgg ccattgcaga ttttcttttt tgtgttacat tgccctttaa gatagcttat
 601 catctcaatg ggaacaactg ggtatttgga gaggtcctgt gccgggccac cacagtcatc
 661 ttctatggca acatgtactg ctccattctg ctccttgcct gcatcagcat caaccgctac
 721 ctggccatcg tccatccttt cacctaccgg ggcctgcccc agcacaccta tgccttggta
 781 acatgtggac tggtgtgggc aacagttttc ttatatatgc tgccattttt catactgaag
 841 caggaatatt atcttgttca gccagacatc accacctgcc atgatgttca caacacttgc
 901 gagtcctcat ctcccttcca actctattac ttcatctcct tggcattctt tggattctta
 961 attccatttg tgcttatcat ctactgctat gcagccatca tccggacact taatgcatac
1021 gatcatagat ggttgtggta tgttaaggcg agtctcctca tccttgtgat ttttaccatt
1081 tgctttgctc caagcaatat tattcttatt attcaccatg ctaactacta ctacaacaac
1141 actgatggct tatattttat atatctcata gctttgtgcc tgggtagtct taatagttgc
1201 ttagatccat tcctttattt tctcatgtca aaaaccagaa atcactccac tgcttacctt
1261 acaaaatagt gaaatgatct tagagaacaa ggacagccat cacagagaac gtctgttttc
1321 aagaacaaca taagcatagt gcaaggagct ccatttccga gctcctaaga aatatgcttc
1381 aaaggtcaaa cattacaaaa gcattagtag tttgtttgtt tgtttttgag actgagtctc
1441 actttatcac ccagactggc gtgcagtggc actatcttgg ctcattgcaa cctctgcctc
1501 ccaggtcagc ctcccaagta gctgggatta caccaccatg cccagctact aaaaatactt
1561 gtatttttag tagagacggg gtttcaccat gttgaccagg ctggtcttga actcctgacc
1621 tcaagtgatc ttccggcctc agcctcccaa agtgctggat tacaggcgtg agccactgag
1681 ccagccagca ttagtaattt ttaaaaacac tttatcagta ttttaaaaat gttaatgcag
1741 gagaaaagat atcacaactc tatggaaaat gacatttcca tttgccttat tgctacttca
1801 agctctttaa atcaccatct tccctatttc
```

//

Sheet 9 of 9 Attorney Docket

REINHOLD COHN 972 3 7109310 08/28 '01 10:08 NO.097 04/04